Charles University
Faculty of Medicine in Hradec Králové

13th INTERNATIONAL MEDICAL POSTGRADUATE CONFERENCE

New Frontiers in the Research of Ph.D. Students

Conference of Medical Schools

Hradec Králové
November 24 – 25, 2016
13th INTERNATIONAL MEDICAL POSTGRADUATE CONFERENCE

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Organized by
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Rector of the Charles University
Tomáš Zima

Hradec Králové
Educational Center of the Faculty of Medicine
Location: University Hospital
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# Organizing Committee

Miroslav Červinka  
Vladimír Palička

Editor:  
Miroslav Červinka

Technical Assistance:  
Petra Malá

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CONFERENCE OFFICE:
The conference office is to be set up for information and registration at the Educational Center in the area of the University Hospital at the following opening hours:

Thursday, November 24, 9:00 – 17:40
Friday, November 25, 8:00 – 16:20

OFFICIAL LANGUAGE:
English

PRESENTATION TIME:
Lecture 15 min
Discussion 5 min

ACCOMMODATION OF PARTICIPANTS:
Hotel Nové Adalbertinum
Velké náměstí 32
500 03 Hradec Králové 3
Dear friends and colleagues,

I would like to welcome you to the 13th International Medical Postgraduate Conference in Hradec Králové. The conference has been progressing well since its inception, and over the years it has turned into a real international meeting. We are proud to welcome participants not only from the Czech Republic and Slovakia, but also from Austria, Georgia, Hungary, Poland, Portugal, Croatia, the Netherlands and the United Kingdom. I personally believe that the position of this conference is well established, and it is a standard part of international activities of our faculty.

There are several reasons for organizing this conference. The first obvious reason is the opportunity to compare achieved results, to present one’s data and learn from others. Nevertheless, we consider this particular meeting of postgraduate students in biomedicine also very important as a tool for international harmonization of Ph.D. studies in the European area. We are very happy that ORPHEUS (Organisation of Ph.D. Education in Biomedicine and Health Sciences in the European System) is an active partner in the organization of this conference.

Another important reason for organizing this meeting is an opportunity for direct personal contacts. Dear participants, take advantage of this occasion not only to learn the news in other medical fields but also to think about the bits of knowledge in other medical areas which can be valuable for you and your postgraduate work. Though there is only “one medicine”, the mutual overlapping of its disciplines can result in great benefits for all.

Those of you evaluated as the best by an expert panel of judges will receive a financial award, yet this should be considered secondary. I am sure that the idea of our meeting is similar to the idea behind the Olympic Games – winning is not the most important thing. Taking part, learning scientific news, and above all meeting new colleagues and friends is of the utmost importance. If we succeed in this, the conference has fulfilled its purpose.

I wish you very successful scientific meeting and enjoyable time in our beautiful city!

Miroslav Červinka
Dean, Faculty of Medicine in Hradec Králové
Charles University
PROGRAMME OVERVIEW

Thursday – November 24, 2016

10:00 – 10:30    Meeting of the Evaluation Committee        Seminar Room 4
10:30 – 11:00    Opening of the Conference                   Main Lecture Hall
11:00 – 13:00    Presentations I (1 – 6)                    Main Lecture Hall
13:00 – 14:00    Lunch                                     Seminar Room 1
14:00 – 15:40    Presentations II (7 – 11)                 Main Lecture Hall
15:40 – 16:00    Coffee Break                             Seminar Room 1
16:00 – 17:40    Presentations III (12 – 16)               Main Lecture Hall

20:00 – 22:00    Social Dinner                            Hotel Nové Adalbertinum

Friday – November 25, 2016

9:00 – 11:00     Presentations IV (17 – 22)                 Main Lecture Hall
11:00 – 11:20    Coffee Break                             Seminar Room 1
13:20 – 14:20    Lunch                                     Seminar Room 1
14:20 – 16:20    Presentations VI (29 – 34)                Main Lecture Hall
16:20 – 17:20    Meeting of the Evaluation Committee      Seminar Room 4

19:30 – 22:00    Social Evening, Awards, Closing Ceremony  Medical Library
                  – Portico Hall
**THURSDAY, NOVEMBER 24**

**Part I**
Chairperson: Aleš Ryška

11:00 **J. Brůha (Plzeň, Czech Republic):** Effective of Selective Portal Vein Embolization and Intraportal Administration of Autologous Stem Cells on the Progression of Colorectal Liver Metastases

11:20 **I. Gullo (Porto, Portugal):** Gastric Carcinoma With Lymphoid Stroma in the Era of the Immune Context and Immunotherapies

11:40 **J. Joos (Dresden, Germany):** Histone H3 Phosphorylation at Serine 28 Regulates Longevity, Heart Function and Heart Morphology in D. Melanogaster

12:00 **N. Kobakhidze (Tbilisi, Georgia):** Genetic Markers of Exfoliation Syndrome in Georgian Population

12:20 **P. Kustán (Pécs, Hungary):** Urinary Orosomucoid as a Potential Diagnostic Marker of Sepsis

12:40 **M. Nováková (Prague, Czech Republic):** Loss of B Cells and Their Precursors is the Most Constant Feature of GATA-2 Deficiency in Childhood Myelodysplastic Syndrome

**Part II**
Chairperson: Emil Rudolf

14:00 **J. Biedermann (Dresden, Germany):** STAT6 Regulates Ephrin-A1 in Human Macrophages, which Influences Cell Mechanics and Migration

14:20 **R. Bower (Hull, United Kingdom):** Head and Neck Cancer: ON-CHIP Culture Provides a Bespoke System for Monitoring the Response of Patient Samples

14:40 **A. Krajčová (Prague, Czech Republic):** Mitochondrial Pathogenesis of Propofol Infusion Syndrome in an In Vitro Model of Human Skeletal Muscle

15:00 **J. Válka (Prague, Czech Republic):** Differential Expression of the Homologous Recombination DNA Repair Genes in Early and Advanced Stages of Myelodysplastic Syndrome

15:20 **K. Vašíčková (Brno, Czech Republic):** Senescence is Modulated by the UPR Response in Ovarian Surface Epithelial Cells
SCIENTIFIC PROGRAMME

Part III
Chairperson: Stanislav Mičuda

16:00  T. Baka (Bratislava, Slovakia): Cardiovascular Remodelling in L-NAME-Induced Hypertension: Effect of Ivabradine

16:20  E. Fárková (Prague, Czech Republic): The Relationship Between Chronotype and Psychosocial Phenomena

16:40  Z. Hanusová (Prague, Czech Republic): Establishment of Permanently Prion Infected Cell Lines for Studies of Prion Strains in Tissue Cultures

17:00  T. Kazda (Brno, Czech Republic): Post Whole-Brain-Radiotherapy Cognitive Impairment and Hippocampal Neuronal Depletion Measured by In Vivo Metabolic MR Spectroscopy

17:20  M. Mešťaník (Martin, Slovakia): Temperament, Character and Biomarkers of Cardiovascular Risk: Interaction Between Neurobiological Model of Personality and Cardiac Autonomic Control

FRIDAY, NOVEMBER 25

Part IV
Chairperson: Martina Řezáčová

9:00  M. Arts (Maastricht, the Netherlands): Matrix Assisted Laser Desorption Ionization Mass Spectrometry Imaging(MALDI-MSI) as a Tool to Map the In Situ Spatiotemporal Distribution of Amino Acids in Tumor Tissue: the Next Generation of Molecular Phenotyping

9:20  M. Elmasry (Liverpool, United Kingdom): Peri-Hepatectomy Pharmacological Induction of the Transcription Factor Nuclear Factor Erythroid 2-Related Factor 2 (Nrf2): A Promising Novel Way of Enhancing Liver Regeneration

9:40  A. Kvernadze (Tbilisi, Georgia): Evolution of West Syndrome in Georgia, Predictors of Outcome

10:00  O. Sobotka (Hradec Králové, Czech Republic): Mitochondrial Functions in Steatotic RAT Liver

10:20  L. Vokálová (Bratislava, Slovakia): Acute Liver Injury: Role of Extracellular DNA Repair

10:40  H. Wilkinson (Hull, United Kingdom): Exploring the Role of Metals in Wound Repair
SCIENTIFIC PROGRAMME

Part V
Chairperson: Jan Laco

11:20 F. Caisberger (Hradec Králové, Czech Republic): Neuroprotective Effect of Oxime Therapy After Sarin Intoxication
11:40 A. Jüngling (Pécs, Hungary): The Effects of Early Environmental Enrichment and PACAP on Monoamine Levels in an Aging Rat Model of Parkinson's Disease
12:00 J. Kosałka (Krakow, Poland): Urinary Markers of Inflammation in Lupus Nephritis Patients
12:20 M. Považan (Vienna, Austria): Fast Ultra-High Resolution 3D Proton MR Spectroscopic Imaging of Human Brain at High Magnetic Fields
12:40 I. Rausch (Vienna, Austria): Lean Body Mass Estimation Form Standard DIXON Based Attenuation Correction in Integrated Positron Emission Tomography/Magnet Resonance Imaging
13:00 P. Šteiner (Plzeň, Czech Republic): Molecular Genetic Analysis of Adenoid Cystic Carcinoma of Salivary Glands

Part VI
Chairperson: Jan Čáp

14:20 R. Adão (Porto, Portugal): Urocortin-2 Attenuates Pulmonary Arterial Hypertension
14:40 P. Krůpa (Hradec Králové, Czech Republic): Experimental Treatment of the Spinal Cord Injury by Human Mesenchymal Stem Cells Derived From Wharton's Jelly (WJ-MSC)
15:00 E. Neis (Maastricht, the Netherlands): Effects of Gut Microbiota Manipulation by Antibiotics on Plasma Amino Acid Levels in Obese Humans
15:20 K. Roženková (Prague, Czech Republic): Genetics and Functional Analysis of Czech Patients With Congenital Hyperinsulinism
15:40 T. Šimurda (Martin, Slovakia): Congenital Afibrinogenemia: Novel Mutation Fibrinogen Martin Leading to Premature Termination Codon in Fibrinogen B Beta-Chain Gene
16:00 M. Vajrychová (Hradec Králové, Czech Republic): Label-Free Quantification of Endogenous Peptides Related to Infectious Inflammation in PPROM Pregnancies
EVALUATION COMMITTEE

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Vladimír Palička  
Vice-Dean for International Relations  
Director of the University Hospital  
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Maastricht University Medical Centre, The Netherlands

Jan Škrha  
Vice-rector for International Relations and Mobility  
Charles University, Prague, Czech Republic
UROCORTIN-2 ATTENUATES PULMONARY ARTERIAL HYPERTENSION

Rui Adão
Department of Physiology and Cardiothoracic Surgery, Cardiovascular Research Centre, Faculty of Medicine, University of Porto, Portugal

Co-authors: P. Mendes-Ferreira, D. Santos-Ribeiro, C. Maia-Rocha, F. Potus, S. Breuils-Bonnet, M. Rademaker, S. Provencher, S. Bonnet, A. Leite-Moreira

Tutor: Carmen Brás-Silva

Introduction and Aims
Pulmonary arterial hypertension (PAH) is a rare and severe syndrome characterized by a progressive remodeling of small pulmonary arteries, leading to elevated pulmonary vascular resistance and right ventricular (RV) failure. Currently, drugs available to treat PAH are restricted to the application of endothelial vasomotor regulatory factors(1). However, the effectiveness of these drugs is limited, and despite significant advances in the treatment of PAH, the long-term prognosis is still poor. RV function is the main predictor of the outcome in PAH and new therapies should protect against RV failure(2).

Urocortin-2 (Ucn-2) is an endogenous vasoactive peptide of the corticotropin-releasing hormone (CRH) family that binds with high affinity to the type 2 CRH receptor (CRHR2), which is expressed abundantly in the cardiovascular system(3). A variety of studies in experimental heart failure (HF) have shown favorable effects of Ucn-2 treatment, which have demonstrated both acute and sustained beneficial actions on cardiac function and remodeling(4,5). Effects reported in healthy volunteers(6) and patients with acute and chronic HF(7,8) have further excited interest in the therapeutic potential of the Ucn-2 in human cardiovascular disease. However, the role of Ucn-2 in PAH and RV failure is still unknown.

Thus, this study aimed to analyze the expression of Ucn-2 in human and experimental PAH, and the effects of human Ucn-2 (hUcn-2) treatment in monocrotaline (MCT)-induced PAH animals. To distinguish cardiac-specific actions from effects on the pulmonary vasculature, hUcn-2 treatment was also studied in an experimental model of pressure overload by pulmonary artery banding (PAB), which results in RV loading without PAH.

Methods
Human Tissue Samples
Human RV samples were collected from patients with and without PAH at the time of cardiac surgery, heart transplantation or autopsy. Samples were categorized according to clinical history and the tricuspid annular plane systolic excursion, and designated as normal RV (NRV) or RV failure (RVF). Blood samples were also collected from PAH patients and age-matched controls.

Animal models and experimental design
Male wistar rats (7-8-weeks old) were injected with MCT (60mg/kg, s.c.) or saline. After two weeks, rats were treated with hUcn-2 (2.5ug/kg, bi-daily, i.p.) or vehicle for 10 days (n=15-22/group), resulting in four groups: Ctrl+vehicle (C); Ctrl+hUcn-2 (CU); MCT+vehicle (M) and MCT+hUcn-2 (MU). At the end of treatment, animals were submitted to exercise testing, echocardiographic and invasive hemodynamic, with subsequent sample collection. Another set of animals was submitted to PAB or sham surgery, and followed the same protocol (n=12-18/group), resulting in three groups: Sham+vehicle (S); PAB+vehicle (B) and PAB+hUcn-2 (BU).
Statistical analysis
Two-way analysis of variance (ANOVA) was used to analyze most parameters. Holm-Sidak’s method for post hoc comparisons between groups and Kaplan-Meier survival analysis (log-rank test) were performed. When appropriate, one-way ANOVA was used. Data are presented as means±SEM. Differences of p<0.05 were considered statistically significant.

Results
RV expression of Ucn-2 and its receptor was increased in MCT animals and patients with RVF. Plasmatic levels of Ucn-2 were increased in experimental and human PAH (Fig.1). hUcn-2 treatment of MCT-animals reduced PAH, resulting in decreased mortality, improved exercise capacity and attenuated pulmonary arterial and RV remodeling and dysfunction. We found attenuation in RV gene expression of hypertrophy and in failure signaling pathways. Moreover, hUcn-2 improved endothelial function (Fig.2). In the PAB model, hUcn-2 attenuated PAB-induced RV hypertrophy (Fig.3).

Figure 1. (A)mRNA expression of Ucn-2 in the RV of patients with NRV and RVF and MCT rats; (B)Representative blots of CRHR2 immunoreactivity in RV of human and rat samples; (C)Ucn-2 levels in the buffy coat of patients with PAH and in plasma of MCT-induced PAH rats. *p<0.05 versus CTRL/NRV.
Discussion

In this work, we evaluated the expression of the Ucn-2/CRHR2 system in experimental and human PAH, and the effects of hUcn-2 treatment in MCT-induced PAH rats. Our results show increased plasma and RV levels of Ucn-2 in both MCT animals and patients with PAH, possibly in response to myocardial stress. This is also the first study that shows an association between the expression of Ucn-2/CRHR2 system with RV dysfunction, suggesting a potential cardioprotective response in this condition.

Additionally, chronic hUcn-2 therapy in experimental MCT-induced PAH significantly attenuated the severity of this disease. Although not all characteristics of PAH are simulated by the MCT model, it shares many characteristics with pulmonary hypertension in humans, including pulmonary vascular remodeling, as well as RV and endothelial dysfunction(2). As Ucn-2 ameliorates most of these parameters, the peptide shows therapeutic potential in this context. Additionally, at 14 days after MCT administration animals exhibit RV hypertrophy and pulmonary flow compromise(9), suggesting that hUcn-2 reverses already established PAH. This is important as PAH in clinical practice is frequently diagnosed in a later stage(1). Furthermore, using a model of pressure loading of the RV without PAH, we also demonstrated that hUcn-2 treatment has direct beneficial effects on RV structure, which is the main predictor of the outcome in PAH.

Conclusions

In conclusion, this study shows for the first time that Ucn-2/CRHR2 signaling may have an important role in PAH and RV dysfunction, given that levels of Ucn-2 and its receptor are altered in human and experimental PAH, and hUcn-2 treatment in a rat model of PAH improves both cardiopulmonary structure and function. These data should encourage further studies to elucidate the underlying mechanisms through which Ucn-2 attenuates the pathophysiology of PAH.

Summary

Pulmonary arterial hypertension (PAH) is a devastating disease and current treatments are limited. Urocortin-2 (Ucn-2) is highly expressed in the cardiovascular system and has shown promising
therapeutic effects in experimental and clinical left ventricular heart failure (HF). Thus, our aim was to analyze the expression of Ucn-2 in human and experimental PAH, and investigate the effects of human Ucn-2 (hUcn-2) administration on right ventricular (RV) function and pulmonary vasculature in rats with monocrotaline (MCT)-induced PAH and RV hypertrophy. Blood and tissue samples were collected from patients with and without PAH and from rats with MCT-induced PAH; and hUcn-2 (5μg/Kg/day i.p. for 10 days) or vehicle was administered to rats subjected to MTC injection or pulmonary artery banding (PAB) (to induce RV overload without PAH). Ucn-2 levels were significantly elevated in the plasma of MCT-injected rats and in buffy coat from PAH patients, and expression of the peptide and its receptor (CRHR2) was increased in the RV of patients and rats with PAH. Ucn-2 treatment in MCT-rats reduced PAH, resulting in decreased mortality, improved exercise capacity and attenuated pulmonary arterial and RV remodeling and dysfunction. Underlying these changes were attenuation in RV gene expression of hypertrophy and failure signaling pathways. In addition, hUcn-2 significantly improved endothelial function. In the PAB model, hUcn-2 treatment attenuated PAB-induced RV hypertrophy. Ucn-2 levels are altered in human and experimental PAH. hUcn-2 treatment attenuates PAH in MCT animals, has direct anti-remodeling effects on the pressure-overloaded RV, and improves pulmonary vascular function.

References


MATRIX ASSISTED LASER DESORPTION IONIZATION MASS SPECTROMETRY IMAGING (MALDI-MSI) AS A TOOL TO MAP THE IN SITU SPATIOTEMPORAL DISTRIBUTION OF AMINO ACIDS IN TUMOR TISSUE: THE NEXT GENERATION OF MOLECULAR PHENOTYPING

Martijn Arts
Email: martijn.arts@maastrichtuniversity.nl

Department of General Surgery, Maastricht University Medical Center+
in Maastricht, The Netherlands


Tutors: Steven W.M. Olde Damink
Co-supervisor: Z. Soons

Document has not been published upon author's request.
CARDIOVASCULAR REMODELLING IN L-NAME-INDUCED HYPERTENSION: EFFECT OF IVABRADINE

Tomáš Baka
E-mail: baka4@uniba.sk

Institute of Pathological Physiology, Faculty of Medicine, Comenius University, Bratislava, Slovak Republic

Co-authors: M. Adamcová, S. Azírová, K. Krajčírovičová, K. Repová, R. Rajkovičová, A. Barta, Š. Paulis, F. Šimko

Tutor: Fedor Šimko

Document has not been published upon author’s request.
STAT6 REGULATES EPHRIN-A1 IN HUMAN MACROPHAGES, WHICH INFLUENCES CELL MECHANICS AND MIGRATION

Jonathan M. Biedermann
E-mail: Jonathan.Biedermann@mailbox.tu-dresden.de

Technische Universität Dresden, Heart Centre Dresden, University Hospital, Dresden, Germany

Co-authors: S. Jellinghaus, G. Ende, M. Kräter, L. Kube, A. Augstein, J. Guck, R.H. Strasser, D.M. Poitz

Tutor: David M. Poitz

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HEAD AND NECK CANCER: ON-CHIP CULTURE PROVIDES A BESPOKE SYSTEM FOR MONITORING THE RESPONSE OF PATIENT SAMPLES

Ruth Bower
E-mail: r.bower@2014.hull.ac.uk

School of Life Sciences, Head and Neck Cancer Laboratory, University of Hull, United Kingdom

Co-authors: V. Green, D. Kuvshinov, N. Stafford and J. Greenman

Tutors: Victoria Green, John Greenman

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EFFECT OF SELECTIVE PORTAL VEIN EMBOLIZATION AND INTRAPORTAL ADMINISTRATION OF AUTOLOGOUS STEM CELLS ON THE PROGRESSION OF COLORECTAL LIVER METASTASES

Jan Brůha
E-mail: bruhaj@fnplzen.cz, jan_bruha@volny.cz

Departement of Surgery, Teaching Hospital and Faculty of Medicine in Pilsen, Czech Republic


Tutor: Vladislav Treska

Document has not been published upon author's request.
NEUROPROTECTIVE EFFECT OF OXIME THERAPY AFTER SARIN INTOXICATION

Filip Caisberger
E-mail: caisbergerf@lfhk.cuni.cz

Department of Toxicology and Military Pharmacy, Faculty of Military Health Sciences, University of Defence, Hradec Králové, Czech Republic

Co-authors: J. Pejchal, J. Misík

Tutor: Jiří Kassa

Introduction
Nerve agents may induce progressive irreversible damage in the central nervous system associated with the dysfunction of irreversibly inhibited AChE and neuronal excitotoxicity and it seems to be largely responsible for persistent profound neuropsychiatric and neurological impairments in the victims of nerve agent exposure [1].

The standard treatment of nerve agent poisoning usually consists of three types of antidotes – anticholinergic agents, oximes and anticonvulsive drugs. Anticholinergic drugs such as atropine sulphate antagonize the muscarinic overstimulation, anticonvulsants such as diazepam are indicated to prevent centrally mediated seizures and secondary brain damage and the oximes disrupt the covalent bond between nerve agent and AChE and restore the physiological function of this enzyme [2]. Many studies have shown there is no potent broad-spectrum oxime able to sufficiently reactivate AChE inhibited by all nerve agents regardless of their chemical structure. HI-6, for instance, is considered to be a relatively strong reactivator of sarin, soman and cyclosarin inhibited AChE but its ability to reactivate AChE inhibited by tabun and less toxic organophosphorus pesticides is of low to moderate values [3]. Trimedoxime or newly synthetized oxime K203, on the other hand, seem to be effective against tabun and pesticides [3]. Since the effort to develop a potent broad-spectrum reactivator has failed so far, another way how to overcome this issue and possibly to increase the reactivating efficacy of antidotes is to combine the oximes in the antidotal treatment [4]. The main purpose of this study was to compare the neuroprotective efficacy of the oxime HI-6 with two oxime mixtures containing HI-6 and trimedoxime or the oxime K-203 in combination with atropine in sarin-poisoned rats. The neuroprotective potential was evaluated using histopathological examination with the help of Fluoro-Jade B fluorochrome used in neuroscience to label degenerating neurons and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay detecting DNA fragmentation during apoptosis.

Methods
Animals were divided into 5 groups (8 rats each): 1) a control group (administered with saline), 2) sarin poisoned group (108 µg/kg – 90% LD50), 3) sarin poisoned group treated with atropine sulphate (21 mg/kg – 5% LD50) and HI-6 (39 mg/kg – 5% LD50), 4) sarin poisoned group treated with atropine sulphate, HI-6 and trimedoxime (7.5 mg/kg – 5% LD50), and 5) sarin poisoned group treated with atropine sulphate, HI-6 and K203 (16.3 mg/kg – 5% LD50). The antidotes were administered 1 min after sarin challenge. Surviving rats were anesthetized by ether vapor 24 hours after intoxication. Samples were subsequently embedded into paraffin and 6µm thick coronary brain sections were cut at level between 3 mm and 4.2 mm from bregma. Selected brain sections were stained with hematoxylin & eosin, Fluoro-Jade B and TUNEL staining. The histological changes were scored using semi-quantitative criteria. Finally, individual animal brain damage scores were calculated representing a sum of histopathological, Fluoro-Jade B and TUNEL scores.
from all tested brain subregions in one animal. The Kruskal-Wallis test with subsequent multiple pairwise comparisons was used to evaluate differences between all groups within particular brain nucleus/subregion. The differences were considered significant when $p \leq 0.05$.

**Results**

The most extensive alterations were found in sarin-poisoned animals without antidotal treatment. Compared to the control group, significantly increased histopathological damage scores in cortex, hypothalamus and piriform cortex ($p = 0.023, 0.001$ and $0.020$, respectively) was measured. In affected animals, cortex and piriform cortex displayed pseudolaminar necrosis in $1$ and $3$ cases, respectively. Hemorrhage was present in one case in hypothalamus. Single cell damage characterized by multifocal degenerative/necrotic changes accompanied with oedema was observed in the remaining positively scored rats. Especially, basolateral nucleus of amygdaloid body, the third and fifth layer of cortex (pyramidal cells), neurons in CA1 (> dentate gyrus > CA3 > CA2) zone of hippocampus, supramammillary hypothalamic nuclei, the third layer of piriform cortex, and medial and dorsolateral thalamic nuclei were markedly impaired.

Administration of antidotal treatment reduced the extent of damage. Any diffuse changes and/or hemorrhage in all groups treated with antidotes were not observed. However, significantly decreased histopathological scores were found in cortex ($p = 0.009$) of animals administered with atropine and HI-6 and in cortex, hippocampus, hypothalamus, and piriform cortex ($p = 0.023, 0.010, 0.009$, and $0.020$, respectively) in rats treated with atropine, HI-6 and K203. Significantly increased Fluoro-Jade B positivity in amygdaloid body, cortex, hypothalamus, piriform cortex, and thalamus ($p = 0.029, 0.032, < 0.001, 0.032$, and $0.029$, respectively) was found in non-treated, sarin-poisoned rats. Diffuse fluorescent patterns were present in $3$ of $5$ surviving rats. In all six regions, maximal Fluoro-Jade B positivity correlated with histopathological findings.

The antidotal treatment markedly reduced brain degeneration marked by Fluoro-Jade B. We found significantly decreased Fluoro-Jade B positivity in hippocampus ($p = 0.017$) of atropine and HI-6 treated rats and in amygdaloid body and hippocampus ($p = 0.038$ and $0.026$, respectively) of animals administered with atropine, HI-6 and trimedoxime. No hemorrhages were observed in both groups. In atropine, HI-6 and K203 treated group, Fluoro-Jade B fluorescence significantly decreased in cortex, hippocampus, piriform cortex, and thalamus ($p = 0.032, < 0.001, 0.032$, and $0.029$, respectively). On the other hand, a small hemorrhage in the hypothalamic region was found. However, Fluoro-Jade B positivity in this area was scored as mild. TUNEL positivity was significantly increased in amygdaloid body, hippocampus and piriform cortex ($p = 0.012, 0.003$ and $0.041$, respectively) of sarin-poisoned animals without antidotal treatment. The distribution of TUNEL staining pattern corresponds to the histopathological and Fluoro-Jade B findings except for hippocampal dentate gyrus.

Atropine and HI-6 treatment did not significantly influence the outcome of sarin intoxication. In atropine, HI-6 and trimedoxime treated group, a decreased amount of TUNEL positive cells was only observed in amygdaloid body ($p = 0.016$). The combination of atropine, HI-6 and K203 decreased positivity in amygdaloid body, hippocampus and piriform cortex ($p = 0.012, 0.003$ and $0.041$, respectively).

**Discussion**

The alterations were particularly profound in cholinceptive subregions and/or subregions sensitive to hypoxic/ischemic stress. This observation is in accordance with generally accepted mechanism of nerve agent-induced acute brain injury and with previously published findings [5]. The distribution of histopathological changes correlated with Fluoro-Jade B and TUNEL staining pattern with one exception. In gyrus dentatus (hippocampus), histopathological findings and Fluoro-Jade B fluorescence showed the highest positivity in the polymorphic layer, whereas TUNEL positivity prevailed in the granular layer. Although both layers display different sensitivity to various stress stimuli [6], the reason of this discrepancy remains uncertain. An explanation may
lie in the limitations of TUNEL method. The method is used to detect DNA fragmentation in apoptotic and necrotic cells. On the other hand, DNA fragmentation occurs also in S phase of cell cycle and the method was reported to produce false positivity in proliferating tissues. Since the subgranular zone of dentate gyrus is considered to be one of the two main neurogenic zones in the adult brain, TUNEL positivity observed in this subregion could possibly represent the damage as well as its regeneration.

Antidotal treatment plays a pivotal role in acute phase of nerve agents’ toxicity [7]. In our model, all three therapeutic regimes mitigated the extent of sarin-induced brain injury. Although we did not find any statistically significant differences among groups treated with oximes, the neuroprotective efficacy of oxime mixtures was slightly higher compared to the group treated with a single oxime. Particularly, the combination of HI-6 and K203 appears to be the most effective to protect experimental animals from acute sarin-induced neuropathological changes in surviving animals. This conclusion corresponds to the previously published results demonstrating the benefit of combinations of oximes for the reactivating and therapeutic efficacy of antidotal treatment of sarin poisoning in rats and mice. Based on the described results, both trimedoxime and K203 do not interfere with HI-6 bioavailability but rather support its action. Therefore, combining the oximes in the antidotal treatment could be a promising step towards a broad-spectrum antidotal treatment of acute nerve agent-exposure regardless of the chemical structure of nerve agent. However, even in HI-6 and K203 group, animals displayed signs of neuronal damage, which tends to progress overtime. Limited therapeutic efficacy is probably related to low blood-brain barrier (BBB) penetration of bispyridinium oximes. Thus, the development and usage of oxime mixtures with better BBB penetration may further increase their ability to counteract the acute toxicity of nerve agents.

Summary
The aim of our study was to compare the ability of two combinations of oximes (HI-6 + trimedoxime and HI-6 + K203) to counteract acute sarin-induced brain damage with the efficacy of antidotal treatment involving single oxime (HI-6) using in vivo methods. Brain damage and neuroprotective effects of antidotal treatment were evaluated in rats poisoned with sarin at a sublethal dose (108 µg/kg i.m.; 90% LD50) using histopathological, Fluoro-Jade B and Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) analysis 24 h after sarin administration. Both combinations of oximes reduce the number of rats that died before the end of experiment compared to non-treated sarin poisoning and sarin poisoning treated with HI-6 alone. In the case of treatment of sarin poisoning with HI-6 in combination with K203, all rats survived till the end of experiment. HI-6 alone was able to reduce sarin-induced brain damage; however, both combinations were slightly more effective. The oxime HI-6 in combination with K203 seems to be the most effective. Thus, both tested oxime combinations bring a small benefit in elimination of acute sarin-induced brain damage compared to single oxime antidotal therapy.

References


PERI-HEPATECTOMY PHARMACOLOGICAL INDUCTION OF THE TRANSCRIPTION FACTOR NUCLEAR FACTOR ERYTHROID 2-RELATED FACTOR 2 (NRF2): A PROMISING NOVEL WAY OF ENHANCING LIVER REGENERATION

Mohamed Elmasry
Email: Mohamed.elmasry@liverpool.ac.uk

Department of Molecular and Clinical Pharmacology, Institute of Translational Medicine
University of Liverpool, Liverpool, United Kingdom

Co-authors: F. Zhang, N. Bird, N. De Bois Brillant, J. Walsh, G. Poston, H. Malik

Tutors: Dr Neil Kitteringham, Professor Chris Goldring, Mr Stephen Fenwick

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THE RELATIONSHIP BETWEEN CHRONOTYPE AND PSYCHOSOCIAL PHENOMENA

Eva Fárková
E-mail: eva.farkova@nudz.cz

Department of Sleep Medicine and Chronobiology, National Institute of Mental Health in Klecany and Third Faculty of Medicine, Charles University, Czech Republic

Co-author: D. Janečková
Tutor: Jana Kopřivová

Introduction and Aims
Circadian rhythms are rhythmically repeating and genetically encoded changes in organisms working on many different levels. They are bound to changes in light/dark cycle and control individual sleep-wake timing, food intake and other processes (Berson et al., 2002). Their disruption may be cause by lifestyle habits such as exposition to artificial light after sunset or shift-work schedule (Hastings, 1997). Disruption of circadian rhythms is considered a risk factor for increased appetite, weight, blood pressure and decreased glucose tolerance or immune response and may result in development of cardiovascular, metabolic or immunological disorders (Karatsoreos, 2014; Walker et al., 2015). From this perspective, individual differences in circadian settings – called chronotypes represent a scientifically interesting area for further research.

There are three chronotypes: morning, evening and intermediate. Their distribution in general population is close to Gaussian and we can find differences between them on many levels (Roenneberg et al, 2007). For example: in reactivity to seasonal changes, tendency to store fat, in psychological characteristics, eating habits and health damaging behaviors or prevalence of lifestyle diseases. In many aspects, individuals with evening type (late chronotype or „owls“) are considered more risky (Hsu et al., 2012; Janečková, 2014; Walker et al., 2015).

In addition, human variability in photosensitivity resulting in a different response to changes associated with seasonal cycles seems to be related to chronotypes. People with late chronotype have three times higher probability to develop SAD (seasonal affective disorder) opposed to larks (morning chronotype). Even if they do not not suffer from SAD, they sleep longer and experience a worse mood during the winter months (Zhang et al., 2015).

The aim of the ongoing project is to describe in detail the human chronotype in the context of psychosocial determinants of health and its frequent complications (obesity, sleep disorders, etc.) in the adult Czech population. This field of chronobiology may greatly enrich clinical practice and disease prevention.

Methods
Data collection has been launched in September 2015 via an internet form. The questionnaire battery is anonymous and contains questions on demography, sleep hygiene and lifestyle as well as scaling questionnaires: MEQ (Morningness – Evenignness Questionnaire Self-Assessment Version), MCTQ (The Munich Chronotype Questionnaire) and SPAQ (Seasonal Pattern Assessment Questionnaire). In this communication we present the results of interim analyses. We used to Shapiro–Wilk test of normality and Pearson correlation for determining a relationship between age and the values in MEQ.
Results
Preliminarily, we can say that the distribution chronotype (MEQ score) in our sample (1071 respondents) corresponds with Gaussian curve (Graph 1). Using the Shapiro - Wilk test for normality data distribution further showed that both sexes divided into different age groups (18-25, 26-49, 50 and over) occupied a collected sample of individuals in terms of chronotype normal distribution (Graph 2).
It was found that there is a relationship between age and scores in MEQ (Pearson correlation), more precisely: the older the individual, the higher the MEQ score, i.e. the more morning chronotype (Tab. 1).

Graph 1: The number of respondents by MEQ score
1 and 5 stand extreme chronotype, 3 is intermediate type.

Graph 2: The distribution of respondents according to the MEQ score and their age.

Table 1: The resulting correlation between age and the MEQ score.

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<th>AGE</th>
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<td>MEQ score</td>
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**Discussion**

So far, no other similar work has been done in the Czech Republic. A previous Czech chronotype study focused on a narrowly defined group - university students. Similarly as in our sample, the distribution of chronotypes among university students according to MEQ data was very close to Gaussian distribution. International studies also report about Gaussian distribution of chronotypes. Moreover, our data revealed a positive association between age and morningness which is also in line with chronotype studies from other countries.

One of the objectives of the study was to determine whether people with an evening chronotype are more often obese and more likely to have diagnosed some of the metabolic and cardiovascular diseases. This will be subject of further data processing and assessing the relationship BMI score and the level of social jet lag, which is a strong indicator of the imbalance of biological and social rhythms. Therefore the further analyses will focus on the relationship between BMI score and the level of social jet-lag, which is a strong indicator of the imbalance of the biological clock and social rhythms. Furthermore, we intend to evaluate the association between MEQ score, social jet-lag and sensitivity to season. We hypothesize that MEQ and SPAQ scores will be related to the severity of social jet-lag and BMI.

**Conclusions**

The study builds on previous work that has been carried out in the Czech Republic and abroad. While also expanding the study chronotype by looking for connections between chronotype and health complications (primarily with obesity and metabolic disorders system). Understanding chronotypes, the associated individual habits and related health issues may help create targeted preventive and therapeutic strategies for diseases related to disorders of circadian rhythms.

**Summary**

Individual differences in circadian settings are called chronotypes (morning, evening, intermediate type). Different chronotypes are linked with differences at many levels and their distribution in general population approximates Gaussian curve. The aim of the ongoing project is to describe human chronotype in the context of psychosocial determinants of adult Czech population, which may have consequences for clinical practice and disease prevention. Data collection has been started in September 2015 via internet forms (a questionnaires battery: MEQ, MCTQ, SPAQ) and it is expected to be finished by the end of 2016. Preliminarily, we can say that the distribution chronotypes in our sample (1071 respondents) based on MEQ score is normal and corresponds to Gaussian curve. Moreover, our data showed that there is a correlation between age and scores in MEQ – higher age is associated with earlier chronotype (higher MEQ score).

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**References**


Introduction and Aims

Gastric cancer (GC) represents a global health concern. Despite advances in prevention, diagnosis and treatment more than 900,000 GC cases are diagnosed per year, causing about 720,000. Molecular target agents have been recently approved, i.e. monoclonal antibodies against HER2 (Trastuzumab) and VEGFR2 (Ramucirumab). Still, these targeted therapies offer a survival advantage of only few months. Among biologic agents, novel cancer immunotherapies can selectively block the cancer evasion of immune surveillance. Phase II/III clinical trials with antibodies directed against CTLA-4 and PD-1/PD-L1 immune inhibitory checkpoints are currently ongoing in GC and are new attractive therapeutic strategies for GC patients [1,2,3,4].

Recently, The Cancer Genome Atlas (TCGA) network proposed a four-tiered molecular classification of GC [5], that could help to guide optimal selection of therapy. This classification defined Epstein-Barr virus (EBV)-positive, microsatellite instable (MSI-high), genomically stable (GS), and chromosomal instable (CIN) tumours. EBV+ and MSI-high GC are two molecular subtypes in which the PD-1/PD-L1 blockade may be particularly beneficial [5,6].

Moreover, a morphological subtype of GC has been associated to MSI-high status and EBV infection, that is Gastric Carcinoma with Lymphoid Stroma (GCLS) [7,8,9]. Hence, the abundant immune infiltrate and the molecular features of GCLS offer an attractive landscape to study tumour immune micro-environment, immune inhibitory checkpoints and their relationship with GC cells. The aim of this study was to analyse the clinico-pathological features, EBV infection, MSI, PD-L1 status and tumour immune microenvironment in GCLS.

Methods

Twenty-four GCLSs, selected from a series of 1088 surgically resected GC patients, were analysed by: RNA in situ hybridisation (EBER) for EBV, PCR/fragment analysis for MSI, and immunohistochemistry (IHC) for cytokeratin (CK) AE1/AE3, CD3, CD8 and PD-L1. PD-L1 immunoreactivity was evaluated separately for tumour immune and epithelial cells. The Immunoreactivity Scoring System (IRS) recently described by Boger C et al was applied [10]. Double immunofluorescence for CK/PD-L1 and CD68/PD-L1 was performed in selected cases. CD3+, CD8+ T cell densities and CD8/CD3 ratio (CD8/CD3R) were calculated both in the tumour centre (TC) and at the invasive front (IF) by digital analysis (Definines®).

A tissue microarray (TMA) was constructed from a control group of 54 non-GCLSs and analysed by IHC for PD-L1 and by EBER.
Results
Figure 1 shows the morphologic characteristics (and diagnostic criteria) of GCSL. Regarding EBV and MSI status, 3 groups were identified: EBV+/microsatellite stable (MSS) (n=16), EBV-/MSI-high (n=4), and EBV-/MSS (n=4).

PD-L1 immunoreactivity was observed at the cell membrane of tumour epithelial cells (IRS>2) in 8/24 GCLSs (33.3%) and in immune stromal cells (≥1%) in 22/24 GCLSs (91.67%) (Fig.2). IRS>2 was restricted to EBV+ (6/16; 37.5%) and MSI-high (2/4; 50%) GCLSs (Fig.3).

Double immunofluorescence, performed in all cases with IRS≥7 (n=5), showed PD-L1 expression in both tumour epithelial (CK+) and stromal immune cells (CD68+). Membranous and strong PD-L1 expression was observed in tumour epithelial cells, whereas cytoplasmic, granular and dotted staining was observed in macrophages/monocytic cells.

Whole-Genome-Sequencing of one PD-L1+ case revealed PD-L1 amplification (the search for PD-L1 amplification in the remaining GCLSs is currently ongoing).

Overall, CD8/CD3R at the IF exceeded CD8/CD3R in the TC (p<0.001). CD8/CD3R was significantly higher (p=0.008) in EBV+ (n=16) than in EBV- (n=8) GCLSs. No significant association was found between CD8+ and CD3+ T cells counts/ratio and MSI status or PD-L1 expression.

By comparison with non-GCLSs, GCLSs were significantly associated with EBV infection (66.7% versus 5.56%, p<0.001) and PD-L1 protein expression (33.3% versus 13.0%, p=0.04). Moreover, GCLSs harbour distinctive clinico-pathological features: younger age (p=0.03), proximal location (p=0.04), indeterminate group of Lauren’s classification (p<0.001), lower lymphatic invasion (p=0.02), lower pTNM stage (p=0.002) and better overall survival (p=0.01).

Fig. 1 GCLS is a particular morphologic subtype of GC, characterised by an abundant lymphoid stroma that widely separates the tumour cells in the entire extent of tumour. Haematoxylin and eosin (H&E).

Fig. 2 PD-L1 expression in GCLS evaluated by IHC staining (clone E1L3N, CST®). Membranous, linear and strong PD-L1 expression was observed in tumour epithelial cells (arrows), whereas cytoplasmic dotted/granular pattern was observed in stromal immune cells (arrowheads). This GCLS case harboured PD-L1 amplification.
Discussion
This study focused on a particular morphological subset of GC, i.e. GCLS, and explored clinico-pathological, molecular characteristics and putative for immunotherapy. As already described in the literature, GCLS is more frequent in young patients, is localised in the proximal stomach, is frequently EBV infected and is associated, per se, to lower pTNM stages and better overall survival. Interestingly, as described in other studies [7,8,9], none of the EBV+ GCLSs was MSI-H and vice-versa, suggesting that EBV positivity and MSI are distinct, and perhaps mutually exclusive pathogenic pathways of GCLS.

To the best of our knowledge, no further studies have been published, so far, addressing the tumour immune microenvironment and PD-L1 immune inhibitory checkpoint in GCLS. In this study, we described a higher CD8/CD3 ratio at IF than TC and in EBV+GCLS compared to EBV-GCLS. GCLSs harboured higher PD-L1 expression, compared to non-GCLS cases. Moreover, PD-L1 expression was restricted to EBV+ and MSI-high cases. All these findings suggest that GCLS is intimately associated to EBV+, MSI-high molecular subgroups and PD-L1 expression. Hence, this morphological subtype, easily recognised by pathologists on routine histological examination, deserves special attention as a predictive factor, to select patients for checkpoint blockade immunotherapies.

Conclusions
GCLSs are characterised by distinctive clinico-pathological features, EBV infection (66.7%), PD-L1 expression (33.3%) and high CD8/CD3R (at the IF and in EBV+ cases). In keeping with the recent molecular data, PD-L1 expression (IRS>2) was restricted to EBV+ and MSI-high cases, reinforcing the potential implications of immunotherapy in these molecular subtypes of GCLS.

Acknowledgements
This work is a result of the project DOCnet (NORTE-01-0145-FEDER-000003), supported by Norte Portugal Regional Programme (NORTE 2020), under the PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF).
This study has been presented in abstract form at the 28th European Congress of Pathology and XXXI International Congress of the International Academy of Pathology, in Cologne, Germany, and was distinguished by the George Tiniakos Award for the best free-paper presentation in gastrointestinal, liver and pancreatobiliary pathology.

Fig. 3. PD-L1 expression (IRS>2) was restricted to EBV+ and MSI-high GCLS.
**Summary**

**Introduction and aims:** Novel cancer immunotherapies, such as PD-1/PD-L1 immune inhibitory checkpoint, can selectively block the cancer evasion of immune surveillance. EBV+ and MSI-high GC are two molecular subtypes in which the PD-1/PD-L1 blockade may be particularly beneficial. A morphological subtype of GC that has been associated to MSI-high status and EBV infection is Gastric Carcinoma with Lymphoid Stroma (GCLS). The aim of this study was to analyse the clinico-pathological features, EBV infection, MSI and PD-L1 status, and tumour immune microenvironment in GCLS.

**Methods:** Twenty-four GCLSs and a tissue microarray constructed from 54 non-GCLSs were analysed by RNA *in situ* hybridisation (EBER) for EBV and IHC for PD-L1. GCLSs were also analysed by PCR/fragment analysis for MSI, IHC for cytokeratin (CK) AE1/AE3, CD3 and CD8 and double immunofluorescence for CK/PD-L1 and CD68/PD-L1. CD3+, CD8+ T cell densities and CD8/CD3 ratio (CD8/CD3R) were calculated both in tumour centre (TC) and at invasive front (IF) by digital analysis.

**Results:** Regarding EBV and MSI status, 3 groups were identified: EBV+/MSS (n=16), EBV-/MSI-high (n=4), and EBV-/MSS (n=4). Overall, CD8/CD3R at the IF exceeded CD8/CD3R in the TC ($p<0.001$). CD8/CD3R was significantly higher in EBV+ than in EBV- GCLSs ($p=0.008$). PD-L1 expression in neoplastic cells was restricted to EBV+ and MSI-high GCLSs. By comparison with non-GCLSs, GCLSs were significantly associated with EBV infection (66.7% versus 5.56%, $p<0.001$) and PD-L1 protein expression (33.3% versus 13.0%, $p=0.04$). Moreover, GCLSs harbour distinctive clinico-pathological features.

**Conclusions:** GCLSs are characterized by EBV infection (66.7%), PD-L1 expression (33.3%) and high CD8/CD3R at the IF and in EBV+ cases. In keeping with the recent molecular data, PD-L1 expression (IRS≥2) was restricted to EBV+ and MSI-high cases, reinforcing the potential implications of immunotherapy in these molecular subtypes of GCLS.

**References**


ESTABLISHMENT OF PERMANENTLY PRION INFECTED CELL LINES FOR STUDIES OF PRION STRAINS IN TISSUE CULTURES

Zdeňka Hanusová
E-mail: zdenka.hanusova@lf1.cuni.cz

Institute of Immunology and Microbiology, 1st Faculty of Medicine, Charles University, Prague, Czech Republic

Tutor: Karel Holada

Introduction
Prion diseases, or transmissible spongiform encephalopathies (TSEs), represent a group of fatal neurodegenerative disorders that affect various mammals including humans. Pathology of prion diseases is closely associated with the conversion of normal cellular prion protein PrP^C to its partially proteolytically resistant PrP^{TSE} isoform, and the prion infection is characterized by subsequent accumulation of PrP^{TSE} in the brain of affected individuals (1). At present, PrP^{TSE} is the only specific biochemical marker of human and animal TSEs. Diagnostic tests are mainly based on the detection of the resistant part of PrP (PrPres) after proteinase K (PK) digestion using various methods, such as Western blot or immunohistochemistry (2). Despite the fact that PrP^C is encoded by the host genome, prions exist in many variants known as prion strains. Within the same species, phenotypically distinct diseases with biochemically distinguishable types of PrP^{TSE} can thus be observed (3). The pathogenesis of prion diseases is widely studied in animal models. However, the cost and complexity of in vivo experiments led to the establishment of a few cell lines that are able to stably propagate infectious prion particles from diverse sources (4). Tissue cultures thus became useful experimental models for studying prion diseases, providing the opportunity to explore different biochemical aspects of these disorders in detail.

Aims
The aim of this study was to prepare new cell culture models for studies of prion strains originating from different hosts using available cell lines which are known to effectively propagate prions in vitro.

Methods
Mouse neuronal cell lines CAD5 and PK1 susceptible for prion infection were incubated with brain homogenates (bh) of mice infected by different prion strains or with normal bh (CD1; negative control). Murine-adapted strains of scrapie (RML; positive control), chronic wasting disease (mCWD), variant Creutzfeldt-Jakob disease (mvCJD) and Gerstmann-Sträussler-Scheinker syndrome (Fu) were utilized for this study. Cells were maintained in culture for 15–20 passages after the infection and their ability to permanently propagate prions was continuously monitored by cell blot (5). Briefly, the cells were grown on plastic coverslips and after reaching the confluence, they were blotted directly onto the nitrocellulose membrane. PrPres-positive cells after PK treatment and denaturation with guanidine isothiocyanate were then identified by immunostaining using the anti-prion antibody. The presence of pathological PrP^{TSE} was evaluated by western blot after specific precipitation of PrP using sodium phosphothungstate (NaPTA) as described previously (6). The difference in PrP^{TSE} amount was assessed by conformation-dependent immunoassay (CDI) utilizing the dissociation-enhanced lanthanide fluorescence immunoassay (DELFIA) measurement and the anti-prion antibody recognizing the PrP^{TSE} epitope that is exposed only after its denaturation. The ratio between the signal of native (N) and denatured (D) samples giving the relative amount of PrP^{TSE} was determined for each infected cell line (7).
Results
As expected, the infection of cells with control RML prions led to the establishment of persistently infected cell lines in both CAD5 and PK1 cells, whereas cells incubated with CD1 remained uninfected. The detection of PrPres after PK treatment by both cell blot and Western blot confirmed that murine-adapted human prion strains mvCJD and Fu were both permanently propagated in CAD5 cells; the infection of CAD5 cells by murine-adapted cervid prions mCWD failed. Only Fu permanently infected PK-1 cells and PrPres after PK treatment was detected by cell blot and Western blot; mvCJD and mCWD prion strains were not propagated. In contrast to Western blot, conformation-dependent immunoassay could be used for PrP\textsuperscript{TSE} detection and for showing differences in the amount of PrP between various prion strains without the need of using PK treatment. The highest D/N ratio and thus relative amount of PrP\textsuperscript{TSE} was measured by CDI for RML-infected cells, whilst the D/N ratio was significantly lower for mvCJD- and Fu-infected cells and differed for CAD5 and PK1 cells.

Discussion
Novel CAD5-derived cell lines chronically infected by mvCJD and Fu became established and are suitable for further studies of these prion strains. Similarly, PK1-derived cell line propagating Fu prions was successfully developed. Further CDI measurements will be conducted to characterize these novel cell lines in more detailed manner. Our attempts to infect cells by murine-adapted cervid prions failed demonstrating peculiar propagation requirements of mCWD strain. Experiments heading towards the establishment of cell culture model to study mCWD are still in progress.

Conclusions
New CAD5/PK1-based model cell cultures for studies of mouse-adapted human prion strains mvCJD and Fu were established. mCWD prions were propagated neither in CAD5 nor in PK1 cells, further experiments are thus planned to investigate this strain and its requirements for efficient propagation in cell cultures.

Summary
Prion diseases (also called transmissible spongiform encephalopathies, TSEs) are neurodegenerative disorders characterized by accumulation of abnormally folded prion protein (PrP\textsuperscript{TSE}) in brain. These diseases can affect mammals including humans and are manifested phenotypically in many variants known as prion strains. The PrP\textsuperscript{TSE} molecule is partially proteolytically resistant, which could be used for its detection after proteinase K treatment by different methods. Traditionally, prions were studied in animals. However, a few cell lines able to effectively propagate prions in vitro recently became available and now represent a valuable tool to investigate prions. This study aimed to establish new cell lines permanently infected by prion strains originating from different hosts. We were able to derive CAD5 and PK1 cell lines stably infected by murine-adapted human prion strains. In contrast, murine-adapted cervid prions were not propagated in the selected cell lines.

Acknowledgements
CAD5 cells were donated by Charles Weissmann, PK1 cells by Peter-Christian Kloehn, RML by Adriano Aguzzi, Fu and mvCJD by Larisa Cervenakova. The study was supported by projects PRVOUK-P24/LF1/3 and SVV260260.
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HISTONE H3 PHOSPHORYLATION AT SERINE 28 REGULATES LONGEVITY, HEART FUNCTION AND HEART MORPHOLOGY IN D. MELANOGASTER

Julius Paul Joos
E-mail: julius.joos@mailbox.tu-dresden.de

Department of Pharmacology and Toxicology, Technische Universität Dresden
Faculty of Medicine, Germany


Tutors: S. Weber, A. El-Armouche

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THE EFFECTS OF EARLY ENVIRONMENTAL ENRICHMENT AND PACAP ON MONOAMINE LEVELS IN AN AGING RAT MODEL OF PARKINSON’S DISEASE

Adél Jüngling
E-mail: junglingadel@gmail.com

Department of Anatomy, University of Pécs, Medical School, Hungary
Tutor: Andrea Tamas, Dora Reglodi

Introduction
The causative therapy of Parkinson’s disease (PD) is still under investigation. One of the well-studied effects of enriched environment and pituitary adenylate cyclase-activating polypeptide (PACAP) is the strong neuroprotective effect. Our research group has shown the protective effects of PACAP in animal models of Parkinson’s disease, Huntington chorea, retinal degeneration and traumatic brain injury. In vitro, we have proven that PACAP is protective against salsolinol-induced neuronal death, an in vitro model of Parkinson’s disease. Our earlier studies have proven that enriched environment is able to reverse some of the deleterious effects of glutamate toxicity on neurobehavioral development and protects the retina against excitotoxicity-induced degeneration. We have previously described the neuroprotective effects of PACAP and enriched environment in Parkinson’s disease in young animals. The aim of our present study is to investigate the protective effects of these factors in one-year-old rats after unilateral 6-OHDA-induced lesion of the substantia nigra, measuring the dopamine (DA) and serotonin (5-HT) levels in the brain.

Methods
Wistar rats were used in our experiment (n=15). Animals were divided into standard (n=7) and enriched groups (n=8) according to their environmental conditions. Animals of the standard group were placed under regular conditions. For environmental enrichment, during the first five postnatal weeks we placed pups in larger cages supplemented with toys, objects, running tunnels and rotating rods of different shape, size and material. Half of the toys were changed daily. In one-year-old animals PD was induced by unilateral injections of 6-OHDA (2 µl 6-OHDA (5 µg/µl)) into the left substantia nigra, control animals received 2 µl physiological saline to the same location. Following the 6-OHDA injections some of the standard-group-animals received 2 µl (1µg/µl) PACAP treatment. The right side of the animals always remained untreated, control side. On the 7th postoperative day the brain of the animals were collected and samples of the substantia nigra were taken with the help of brain matrix. Dopamine and serotonin levels were measured by HPLC-Q Exactive orbitrap MS system in control, 6-OHDA and 6-OHDA+PACAP injected rats of the standard and enriched environment.

Results
Physiological saline did not cause any significant decrease in DA levels in either of the animals. The substantia nigra of the 6-OHDA-treated standard and enriched animals showed significantly lower DA levels compared to the saline-treated animals of the same groups. Consistent with our previous studies in young animals the PACAP treatment could increase the DA levels with 15% after 6-OHDA induced lesion. The early environmental enrichment didn’t have any protective...
effects in our present experiment. No significant differences could be observed regarding the serotonin levels of the substantia nigra.

Conclusions
Although the protective effect of early postnatal environmental enrichment is described in young animals, we could not prove it in our experiment on aging animals. However, similarly to younger animals PACAP could restore the decrease of DA levels, which could play a role in its neuroprotective effect in Parkinson's disease.

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POST WHOLE-BRAIN-RADIOThERAPY COGNITIVE IMPAIRMENT
AND HIPPOCAMPAL NEURONAL DEPLETION MEASURED BY IN-VIVO
METABOLIC MR SPECTROSCOPY

Tomáš Kazda
E-mail: tomas.kazda@mou.cz

Department of Radiation Oncology, Masaryk Memorial Cancer Institute,
Brno, Czech Republic

Co-authors: P. Pospisil, R. Jancalek, P. Slampa

Tutor: Radim Jancalek

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GENETIC MARKERS OF EXFOLIATION SYNDROME IN GEORGIAN POPULATION

Nino Kobakhidze
E-mail: ninokobakhidze@gmail.com

David Tvildiani Medical University, Tbilisi, Georgia
Chichua Medical Center Mzera, LLC, Tbilisi, Georgia

Co-authors: G. Chichua, T. Aung T, CC Khor
Tutor: Sergo Tabagari

Purpose
The aim of this study was to identify susceptibility genes/loci for Exfoliation Syndrome and Exfoliation Glaucoma by a case control cohort study approach in Georgian population.

Materials and Methods
Eighty-three patients aged over 50 with XFS and 103 normal subjects over 60, having no clinical evidence of exfoliation material in anterior segment structures of the eye were included in this study. After signing an informed consent patients underwent venous blood sampling. Case control association study was performed by two-stage Genome-wide association study (GWAS). The genetic regions identified to harbor XFS susceptibility genes, were subsequently genotyped for the 750,000 Single Nucleotide Polymorphisms (SNPs) on Illumina chips in unrelated cases of exfoliation syndrome and controls. For replication, the Sequenom MassArray platform was used to test SNPs that were highly associated with the disease ($P < 1 \times 10^{-5}$).

Results
Principal component analysis of the Georgian XFS cases and controls showed the cases and controls to be well-matched genetically with minimal evidence of population stratification. Furthermore, the genome-wide data showed the Georgian XFS case-control collection to have an ancestry distinct from the other Northern, Western, Eastern, and Southern European collections. In Georgian, the frequency of LOXL1 rs3825942 was 95.8% in exfoliation cases and 84% in controls, $P = 0.0001$, OR=4.65. The frequency of CACNA1A rs4926244 was 20% in XFS patients and 17% in controls, OR =1.19 ($P = 0.4$ in Georgia and $P < 10^{-10}$ for global meta-analysis). The genetic effect sizes observed by us in Georgia were consistent with the data found in previously published studies for LOXL1 and CACNA1A.

Discussion
Exfoliation syndrome is an age-related disorder characterized by excessive production and progressive accumulation of extracellular fibrillar material in different structures of the eye. XFS is the most common identifiable cause of open-angle glaucoma and a very frequent cause of serious complications during cataract surgery (Ritch R, 2003). XFS patients are found to have three high-risk SNPs of LOXL1 gene on chromosome 15: rs2165341, rs10488661 and rs3825942 (Thorleifsson, G. 2007). Lysil oxidase is a member of copper-dependent enzymes, which plays role in genesis, stabilization and remodeling of elastic fibers. (Liu et al., 2004; Oleggini, 2007). It catalases deamination of lysil remnants of tropoelastin, which is the most important reaction in elastogenesis (Ursula Schlötzer-Schrehardt et al., 2012). LOXL1 mutation causes overproduction of fibrillar material and its deposition in the anterior segment structures of the eye. SNP rs4926244 of the CACNA1A gene has also recently been linked to development of XFS. It encodes @ subunit of voltage dependent type P/Q calcium channels (Aung et al., 2015). These channels are involved in transmembrane transport of calcium
and therefore in generation and transmission of electric signals. It was found that exfoliation material contains high concentrations of calcium (U Schlötzer-Schrehardt, 2001). It is also well known that fibrillin uses calcium for making stable aggregates. Interestingly, different SNPs have causative role in different populations. In Georgian population LOXL1 rs3825942 and CACNA1A rs4926244 are associated with high risk of disease development. The fact, that risk alleles are often “flipped”, implies that genetic architecture of XFS is very complex and needs further clarification.

Conclusion
SNPs of the LOXL1 and CACNA1A genes are associated with XFS in the Georgian population. Our data are consistent with previously reported findings in European and Asian populations. Although, further studies are needed to provide more insight into pathogenesis of the disease.

References
URINARY MARKERS OF INFLAMMATION IN LUPUS NEPHRITIS PATIENTS

Joanna Kosalka  
E-mail: joanna.kosalka@gmail.com

2nd Department of Internal Medicine Unit of Allergy and Clinical Immunology Jagiellonian University, Medical College Kraków, Poland

Co-authors: J. Musiał, B. Jakiela

Tutors: Jacek Musiał, Bogdan Jakiela

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MITOCHONDRIAL PATHOGENESIS OF PROPOFOL INFUSION SYNDROME IN AN IN VITRO MODEL OF HUMAN SKELETAL MUSCLE

Adéla Krajčová
E-mail: adela.krajcova@lf3.cuni.cz

Laboratory of Metabolism and Bioenergetics, Third Faculty of Medicine, Charles University, Prague, Czech Republic

Co-author: P. Waldauf

Tutors: Michal Anděl, František Duška

Document has not been published upon author's request.
EXPERIMENTAL TREATMENT OF THE SPINAL CORD INJURY BY HUMAN MESENCHYMAL STEM CELLS DERIVED FROM WHARTON’S JELLY (hWJ-MSC)

Petr Krůpa
E-mail: petrkrupa@centrum.cz

Department of Neurosurgery, Faculty of Medicine in Hradec Králové, Charles University and Institute of Experimental Medicine v.v.i., The Czech Academy of Sciences, Czech Republic

Co-authors: T. Amemori, I. Vacková, J. Růžička, K. Zavišková, J. Dubišová, P. Jendelová

Tutor: Svatopluk Řehák
Tutor specialist: Pavla Jendelová

Introduction
Spinal cord injury (SCI) remains one of the most physically, psychologically and socially debilitating medicine problems in the world. So far there is no specific or effective primary treatment yet available. Injury to the spinal cord results in motor and sensitive deficit, which severity depends on mechanism of the injury (primary damage) and on endogenous processes following the direct trauma in hours and days (secondary damage). While direct trauma can be hardly affected, modulation of the local inflammatory response, scavenging reaction and production of growth factors can result in better regeneration of the tissue.

In recent decade a variety of stem cells were used to affect the process of cell apoptosis, demyelination and axonal degeneration. Mesenchymal stem cells (MSCs) are a type of multipotent progenitor cells that can be differentiated into several cell types of mesodermal origin including osteocytes or cardiomyocytes. Major sources of MSCs are bone marrow and umbilical blood, but they are also found in adipose tissue or Wharton’s Jelly surrounding the umbilical vessels [1]. Their beneficial effect in spinal cord injury treatment is due to their ability to secrete trophic factors, which can affect secondary processes occurring after SCI, promoting axon regeneration, angiogenesis and reduce inflammatory cell activation and glial scar formation.[2]

Aims
The aim of the study was to determine, whether the human mesenchymal stem cells derived from Wharton’s Jelly could improve the functional outcome of the rat with ischemic-compression spinal cord injury. Secondary purpose was to evaluate, if the treatment is dose – responsive – e.g. if the repetition or higher doses of transplanted stem cells would result in better recovery.

Methods
Experiment was performed on 10-weeks-old male Wistar rats with the body weight 300g ±15g. Ballon-induced spinal cord injury model was utilized as a ischemic-compression model of SCI. Rats were divided into five groups. Group A (single treatment of 0.5mil hWJ-MSC), Group B (single treatment of 1.5mil hWJ-MSC), Group C (repeated treatment of 0.5mil hWJ-MSC), Group D (repeated treatment of 1.5mil hWJ-MSC) and Group E (control group receiving saline). Seven days after SCI, hWJ-MSC (5 x10⁵ or 1.5 x10⁶/ 50 ml saline) were injected by the lumbar puncture into the subdural space through the L5-L6 intervertebral space. Groups with repeated treatment received another injection 14 and 21 days after SCI. Control rats were injected with 50ml of saline into the L5-L6 interspinous space.
Through the experiment the behavioural assessment was regularly performed. Locomotor function was tested by Basso, Beattie, and Bresnahan (BBB) open field test, flat-beam test and rotarod test every week after lesion.

Eight weeks after spinal cord injury all rats were perfused with 4% paraformaldehyde in PBS and their spinal cords were removed, embedded in paraffin and cut into cross sections. Samples were stained with Cresyl violet Luxol to visualise white and grey matter, with GAP43 to analyse axonal sprouting and with GFAP to recognize the glial scar.

**Results**

1. **Behavioural testing:**
   Group with single treatment of 1.5 mil hWJ-MSC and groups with repeated treatment of 3x0.5mil or 3x1.5mil hWJ-MSC recovered significantly better than control (two-way RM ANOVA, Treatment p<0.05). Treatment with single dose of 0.5 mil hWJ-MSC didn’t show any significant difference compared to control. Testing of advance motor function and limb coordination by flat beam test and rotarod test showed no or minimal differences between the treated groups and control.

2. **Histological assessment:**
   Grey and white matter sparing was measured as a remained tissue on cross section areas of the treated animals compared to the control group. Significant difference in preserved grey matter achieved only group with 3x1.5 mil hWJ-MSC (two-way RM ANOVA, Treatment p<0.05). No significant difference in preserving white matter was observed. Glial scar around the main cavity was measured and counted as a percentage of whole cross section tissue. Astrogliaosis was significantly lower in higher single dose – 1x1.5mil hWJ-MSC and in both repeated doses – 3x0.5mil and 3x1.5mil hWJ-MSC. Axonal sprouting was counted as a number of fibers in GAP43 staining. There was significant higher number of GAP43+ fibers in all treated groups with exception of 1x0.5mil hWJ-MSC (one-way ANOVA, Treatment p<0.05)

**Conclusions/Summary**

In the presented experimental study we concluded, that treatment by single dose of 0.5 mil. hWJ-MSC (group A) had no or minimal benefit on recovery from the spinal cord injury in rat model. We also proved that higher doses and repeated treatment (group B,C,D) of hWJ-MSC significantly improved overall functional outcome. Though there was no difference between the groups B,C and D in behavioural testing, group D had significantly higher number of GAP43+ fibers and had significantly larger area of spared grey matter in the centre of the lesion. These findings can lead to hypothesis that in long term recovery (> 9 weeks) rats in group D would have higher potential to improve than rats in group B and C.

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URINARY OROSOMUCOID AS A POTENTIAL DIAGNOSTIC MARKER OF SEPSIS

Péter Kustán
E-mail: peter.kustan89@gmail.com

Department of Laboratory Medicine, Department of Anaesthesiology and Intensive Therapy, Szentágothai Research Centre, University of Pécs, Hungary

Co-authors: Z. Horváth-Szalai, B. Szirmay

Tutors: Tamás Kőszegi, Diána Mühl

Introduction and Aims
Although the sepsis syndrome has been known since the ancient times it still remains a challenging healthcare problem nowadays. To improve disease outcome, early goal-directed therapy is necessary therefore prompt diagnosis is of utmost importance [1]. Laboratory parameters may be extremely useful in rapid clinical decision making and also in monitoring of sepsis. As an acute phase protein the serum levels of orosomucoid (ORM) can increase up to two-threefold during systemic inflammation [2]. Many studies investigated serum ORM but only scarce data are available on urinary ORM (u-ORM), however, ORM can also be found in the urine, as well. A few former studies reported slight elevation of u-ORM levels in diseases associated with chronic inflammatory activation. In spite of promising data, automated u-ORM assay is unavailable yet. For daily clinical utilization we developed and validated an immune turbidimetric assay for u-ORM measurements on a fully automated clinical chemistry analyzer. Our further aim was to determine reference range for u-ORM in healthy individuals. Furthermore our goal was to monitor u-ORM levels in critically ill patients hypothesizing that u-ORM is a useful marker of sepsis. We investigated the diagnostic and prognostic ability of u-ORM, too.

Methods
A particle-enhanced turbidimetric assay was validated for Cobas 8000/c502 analyzer to measure u-ORM levels by using orosomucoid immunoparticles (Dako A/S, Glostrup, Denmark). In order to determine a reference range for u-ORM, healthy volunteers (n=72) were recruited between the age of 10 and 60 years. We monitored u-ORM levels in patients with systemic inflammatory response syndrome (SIRS, n=13) and in septic patients (n=39) from our intensive care unit (ICU). To exclude the influence of comorbidities on u-ORM concentrations, a group of volunteers (n=30) served as matched-control group to ICU patients (similar in age, sex and medical history). Our study was approved by the Regional Ethics Committee of the University of Pécs, (no. 4327.316-2900/KK15/2011). Our patients were fully informed and written consent was obtained from all. Spontaneous random urine samples and venous blood were simultaneously obtained from the participants. To avoid the influence of urine volume, u-ORM concentrations were referred to urinary creatinine (u-CREAT) and expressed as u-ORM/u-CREAT (mg/mmol). Statistical analyses were performed by IBM SPSS Statistics for Windows, Version 22. For comparison of our groups Kruskal-Wallis or Mann-Whitney U tests were carried out. Receiver operating characteristic (ROC) curves were used for analyzing the diagnostic and predictive values. Our data are presented as median and interquartiles.

Results
Our new turbidimetric approach was set up to be fast (10 minutes) and sensitive with a detection limit of 0.02 mg/L. The working range of our assay was 0.16-5.25 mg/L. The intra- and inter-assay
imprecision as CV%, and also the inaccuracy were <5%. Within 10 to 60 years, a reference range for u-ORM/u-CREAT was found to be 0.08 (0.05-0.15) mg/mmol. Compared to these data a slightly (p<0.01) increased u-ORM/u-CREAT was found in the volunteers with comorbidities 0.2 (0.1-0.3) mg/mmol. On ICU admission, both SIRS and septic patient groups showed strongly elevated u-ORM/u-CREAT values compared to matched-controls (p<0.001). Furthermore, significantly higher (p<0.001) u-ORM/u-CREAT levels were found in sepsis than in SIRS (Table 1). The area under the ROC for distinguishing SIRS from sepsis was found to be 0.954 for u-ORM/u-CREAT, superior to se-ORM and hs-CRP (Figure 1). A cut off value for u-ORM/u-CREAT with 94.7% sensitivity and 90% specificity could be set at 6.75 mg/mmol to discriminate SIRS from sepsis. U-ORM/u-CREAT levels did not change during the 5-day follow-up period. We found no differences between the survivor and non-survivor septic patients (Figure 2). Significant (p<0.001) positive correlation between u-ORM/u-CREAT and se-ORM (ρ=0.693) and hs-CRP (ρ=0.600) levels were described.

Table 1: Patients’ characteristics

<table>
<thead>
<tr>
<th></th>
<th>Reference group n=72</th>
<th>Matched-controls n=30</th>
<th>SIRS n=13</th>
<th>Sepsis n=39</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females, n (%)</td>
<td>37 (51.4)</td>
<td>14 (46.7)</td>
<td>4 (30.8)</td>
<td>15 (38.5)</td>
<td>N/A</td>
</tr>
<tr>
<td>Age, (years)</td>
<td>23 (14-45)</td>
<td>58 (49-69)</td>
<td>62 (54-70)</td>
<td>67 (57-76)</td>
<td>N/A</td>
</tr>
<tr>
<td>Cardiovascular disease, n (%)</td>
<td>0 (0)</td>
<td>19 (63.3)</td>
<td>10 (76.9)</td>
<td>26 (66.7)</td>
<td>N/A</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>0 (0)</td>
<td>6 (20.0)</td>
<td>3 (23.1)</td>
<td>10 (25.6)</td>
<td>N/A</td>
</tr>
<tr>
<td>Pulmonary disease, n (%)</td>
<td>0 (0)</td>
<td>2 (6.7)</td>
<td>6 (46.2)</td>
<td>14 (35.9)</td>
<td>N/A</td>
</tr>
<tr>
<td>hs-CRP, (mg/L)</td>
<td>0.6 (0.3-1.5)</td>
<td>1.0 (0.5-2.2)</td>
<td>92.4 (56.4-227.9)</td>
<td>228.5 (138.6-336.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>u-ORM/u-CREAT, (mg/mmol)</td>
<td>0.08 (0.05-0.15)</td>
<td>0.20 (0.10-0.30)</td>
<td>2.10 (0.70-6.40)</td>
<td>19.20 (11.40-32.80)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Figure 1: ROC analysis for distinguishing SIRS from sepsis

Figure 2: Monitoring of u-ORM/u-CREAT levels in sepsis
Discussion
Our fully automated turbidimetric approach was described to be fast, sensitive and precise therefore it is ideal for routine u-ORM measurements. We determined a reference range for u-ORM/u-CREAT to be similar to others [3]. We found slightly elevated u-ORM levels in volunteers with comorbidities, which is in agreement with previous findings on diabetic patients [4]. This preclinical elevation is considered to be the result of chronic ongoing low-grade inflammation. Furthermore, we observed extremely increased u-ORM excretion in severe systemic inflammation compared to controls. We described an early and relevant u-ORM/u-CREAT elevation in sepsis with promising diagnostic performance to distinguish SIRS from sepsis. Although ORM/u-CREAT seems to be sensitive and early marker of sepsis with good diagnostic ability, it is unable to predict disease severity and mortality. The pathomechanism of u-ORM excretion is not well understood and the increased se-ORM level itself may not explain that alone. Alteration in kidney function, probably both glomerular and tubular dysfunctions may be involved. Furthermore, a possible local renal production due to systemic manifestation of inflammation is also suspected. The good correlations between inflammatory markers and u-ORM/u-CREAT support the hypothesis that elevated u-ORM is mainly due to systemic inflammation.

Conclusions
Our highly sensitive u-ORM assay is ideal for routine clinical utilization to measure u-ORM as a possible novel marker of inflammatory activity. U-ORM showed a considerably early elevation in sepsis with promising diagnostic performance in discriminating SIRS cases from true sepsis. Consequently, non-invasively obtained urine could be a possible alternative to blood sampling in diagnosis of sepsis, and u-ORM/u-CREAT might be suitable as a complementary marker of sepsis and could help clinicians in proper decision making.

Summary
We developed a fully automated u-ORM assay, which can be a novel diagnostic tool in management of severe systemic inflammation.

References


EVOLUTION OF WEST SYNDROME IN GEORGIA, PREDICTORS OF OUTCOME

Ana Kvernadze
E-mail: anano_kvernadze@yahoo.com

David Tvildiani Medical University
Neuroscience Department at M. Iashvili Children's Central Hospital, Tbilisi, Georgia

Tutor: Nana Tatishvili

Introduction. Aims
Infantile spasms is an age-specific devastating epileptic encephalopathy of early infancy, which is characterized by the combination of distinctive unique seizure type, which is accompanied by specific electroencephalographic pattern known as hypsarrhythmia and delay or arrest of psychomotor development. This is a prospective observational study about infantile spasms and predictors of outcome. This syndrome hasn’t been thoroughly investigated in Georgia and our purpose is to assess the evolution of West syndrome and its relation to patient characteristics, to study etiological peculiarities and its relation to the long-term outcome, to find out if time between initiation of spasms and treatment make the influence on the long-term outcome (lead time to treatment).

Methods
We evaluated 28 patients (16 male, 12 female) with infantile spasms at M. Iashvili Children Central Hospital, Institute of Neurology and Neuropsychology and I. Tsitsishvili Children New Hospital, Tbilisi, Georgia. Mean age - 6-7 months. Inclusion criteria were newly diagnosed patients, who met definition of West syndrome (IS, abnormal EEG), written informed consent of parents/ caregivers. We collected birth, family and seizure detailed history. All patients were examined neurologically, investigated with prolonged sleep and awake video-EEG, brain MRI, developmental testing (Bayley scales of infant and toddler development, III edition, ASQ - age and stage questionnaire) was done at admission. Spasm diary was given and filled by every parent/caregiver. Seizures were recorded on video and phenomenology of infantile spasms was studied. All patients were treated with adrenocorticotropic hormone (ACTH), hydrocortisone or antiepileptic drugs. Second and third years we are planning to do follow-up and assess: evolution of infantile spasms into other epilepsy (e.g. Lennox- Gastaut syndrome, symptomatic focal epileptic syndromes, etc.), treatment options with ACTH/antiepileptic drugs, electroencephalographic evolution after treatment and neurodevelopmental testing.

Results
Patients’ age below than 6 months, at the onset of spasms, was in 12 (42%) and above than 6 months in 16 children (58%). Etiologically symptomatic IS were present in 22 (79%) of cases. Among them - corpus callosum hypogenesis 6, hydrocephaly 6, lissencephaly 1 and various 9 cases. Cryptogenic in 6 (21%) cases. Symmetrical spasms were most common (82%). Most of the patients had spasms in cluster (85%). Hypsarrhythmia on EEG was seen in 20 (72%) patients, modified (atypical) hypsarrhythmia in 4 (14%) and other changes also in 4 (14%) patients. All patients were treated. After 5-8th injection with ACTH therapeutic response was satisfactory in 93%. Developmental delay was seen in 25 patients (89%), normal development -3 patients (11%). One patient died.
Discussion
Neurodevelopmental outcome, due to underlying etiology, is unfavorable. Among prognostic factors, the treatment lag and duration of spasms have been recognized as being significant. In our study number of patients isn’t much, though preliminary findings are consent to the other author’s studies. In symptomatic cases prognosis is unfavorable. Adrenocorticotropic hormone remains as a first choice treatment option.

Conclusion
Prognosis remains unfavorable and poor. Developmental assessment was done at the time of spasms initiation, most of the patients have rehabilitation course. Cessation of spasms was more likely in infants given hormonal treatments. This study is still in the process. We are planning to do clinical, EEG and neuropsychological follow-up one and two years after first assessment.

Summary
West syndrome is the object of a number of studies aimed at understanding the complex relationships between an epileptic disorder and neurodevelopment. Despite of such a huge interest and plenty of researches around West syndrome there still are too many questions that need to be addressed: unique seizure type, the phenomenon of spontaneous remission, pathophysiology, to date model of infantile spasms isn’t known yet, evolution of Infantile spasms, what factors determine the outcome, treatment remains problematic (3).
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TEMPERAMENT, CHARACTER AND BIOMARKERS OF CARDIOVASCULAR RISK: INTERACTION BETWEEN NEUROBIOLOGICAL MODEL OF PERSONALITY AND CARDIAC AUTONOMIC CONTROL

Michal Mešťaník
E-mail: mestanik@jfmed.uniba.sk; mestanik@gmail.com

Department of Physiology and Biomedical Center Martin, Comenius University in Bratislava, Jessenius Faculty of Medicine in Martin, Martin, Slovak Republic

Co-authors: Z. Višňovcová, A. Mešťaníková

Tutor: Ingrid Tonhajzerová

Introduction
It has been known for several decades that people with certain personality traits and behavioural characteristics are more prone to cardiovascular diseases (CVD); in particular, type A personality characterized by competitiveness, time urgency and hostility was the most widely used construct related to cardiovascular morbidity (Booth-Kewley and Friedman, 1987). Recently, type D (distressed) personality associated with negative affectivity, social inhibition, anxiety and depression has been extensively studied as a risk factor for adverse cardiovascular outcomes (Cao et al., 2016). From this context, the role of personality and behaviour in the etiopathogenesis of CVD is universally recognized. However, the mechanisms underlying this relationship are still not fully understood. Therefore, we aimed to study the interaction between personality traits and physiological cardiac autonomic regulation at rest and in response to mental stress using a modern concept of personality - Cloninger’s neurobiological model and complex heart rate variability (HRV) analysis by conventional linear methods and novel nonlinear parameters in young healthy adults.

Methods
The studied group comprised 40 university students (20 women, age: 22.9±0.1 yr., BMI: 22.0±0.4 kg/m²) with excluded effect of underweight, overweight, recent acute illness, history of chronic cardiovascular, respiratory, endocrine, neurological, infectious diseases, mental disorders, smoking and use of medicaments and substances which could affect autonomic nervous system (e.g. alcohol, caffeine at least 12 h before the examination).

The examinations were performed under standard conditions in a sound-attenuated room with minimization of stimuli, between 9.00 a.m. – 12.30 p.m. After 15 min required to avoid a potential stress effect of laboratory environment, the continuous recording of ECG signal (DiANS PF8, Dimea, Czech Republic) was performed during stress assessment protocol: baseline (P1), Stroop test (P2), rest (P3), mental arithmetic test (P4), rest (P5), negative emotional stimulus (P6), and rest (P7). The duration of each period was 6 min. The RR-intervals were derived from ECG-recordings and following parameters of HRV were evaluated: RR-interval, spectral power in high frequency band of HRV (HF-HRV; reflecting cardiac vagal control), nonlinear HRV analysis - symbolic dynamics indices 0V% (cardiac sympathetic regulation) and 2LV% (cardiac vagal control), parameters indicating HRV complexity – normalized index of complexity (NCI) and normalized index of unpredictability (NUPI).

Personality traits were assessed according to Neurobiological model of personality, which integrates the neurobiological and psychological aspects of personality (Cloninger, 1999). Slovak version of the Temperament and Character Inventory – Revised was used. This inventory (240 items) allows to quantitatively assess the temperament and character dimensions
of personality. Four temperament dimensions are considered to reflect functions of central neurotransmitters: novelty seeking (associated with behavioural activation and function of dopamine), harm avoidance (associated with behavioural inhibition and function of GABA and serotonin), reward dependence (noradrenaline and serotonin), and persistence (glutamate and serotonin). The temperament traits are supposed to be largely stable and inherited. Three character dimensions associated with social-learning and education (acquired dimensions) are self-directedness, cooperativeness, and self-transcendence.

Results
RR-interval was significantly shorter and lnHF-HRV and 2LV% were significantly lower in response to both cognitive (T2, T4) and emotional (T6) tasks (RR-interval: p<0.001 for T2 and T4, p<0.01 for T6; lnHF-HRV and 2LV%: p<0.01 for T2 and T6, p<0.001 for T4). Index Ov% significantly increased in response to cognitive mental tasks (T2, T4; both p<0.001). Parameters NCI and NUPI were significantly lower during cognitive mental tasks (T2: both p<0.001, T4: NCI p<0.05, NUPI p<0.01). During recovery periods (T3, T5, T7), all the parameters returned to baseline values.

Correlation analysis revealed significant negative correlation between harm avoidance and Ov% during mental arithmetic task (r=-0.356, p=0.024) and significant positive correlation between cooperativeness and resting NCI and NUPI (r=0.391, p=0.013; r=0.383, p=0.015, respectively).

Discussion
We found that applied mental stressors were suitable to evoke a shift in dynamic autonomic sympathovagal balance assessed by linear and nonlinear HRV analysis. The nonlinear methods of HRV analysis revealed associations between personality traits and cardiac autonomic regulation in young healthy subjects, while conventional linear analysis was not sensitive to this relationship. The complex cardiac autonomic regulation is maintained by an integrated system of numerous mutually interconnected cortical and subcortical nervous centres which is known as central autonomic network (Benarroch, 2007). Existence of functional neural connections between cortical areas, limbic system and brainstem nuclei can result in fact, that parameters of HRV may reflect regulation of various psychophysiological processes, including personality and behaviour. In our study, subjects with more expressed behavioural inhibition marked by harm avoidance showed lower cardiac sympathetic reactivity to mental stressor. This interaction could be related to inhibitory GABA-ergic neurotransmission affecting the central processing of stress response (Nuss, 2015). Importantly, low stress reactivity has been recently considered to be a risk factor for CVD similarly as exaggerated physiological reactions (Lovallo, 2011). Interestingly, our study also revealed the association between acquired character trait and autonomic cardiac regulation. Participants with greater cooperativeness showed higher complexity and unpredictability of HRV, which are considered as novel biomarkers of effective physiological neuro-cardiac control and were found to be decreased under pathological conditions (e.g. in attention deficit/hyperactivity disorder, Tonhajzerova et al., 2016). Thus, the interaction between personality and autonomic regulation seems to be bidirectional with temperament personality traits being based on neurobiological properties of nervous system and vice versa autonomic nervous functions being affected by character dimensions of personality.

Conclusions
Application of novel nonlinear analysis of heart rate variability and Neurobiological model of personality seems to be a promising method to study the effect of personality on autonomic cardiac regulation. This study could help to understand the interaction between personality characteristics, autonomic nervous system, and risk of cardiovascular diseases. After more detailed study of the underlying mechanisms and long-term effects of personality traits, clinical application could include an improved assessment of the cardiovascular risk, individual adjustment
of prevention, diagnostics and treatment of CVD, and potential effect of intervention in character personality dimensions on future health outcomes.

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Summary
It has been known for several decades that people with certain personality traits and behavioural characteristics are more prone to cardiovascular diseases (CVD). However, the mechanisms underlying this relationship are still not fully understood. Therefore, we aimed to study the interaction between personality traits and physiological cardiac autonomic regulation at rest and in response to mental stress using a modern concept of personality - Cloninger’s neurobiological model and complex heart rate variability (HRV) analysis by conventional linear methods and novel nonlinear parameters in young healthy adults. The ECG recordings in studied group of 40 healthy young adults were performed during stress assessment protocol: baseline, Stroop test, rest, mental arithmetic test, rest, negative emotional stimulus, and rest. The dimensions of personality – temperament - neurobiological inherited traits and character - social acquired traits were quantitatively assessed using the Slovak version of the Temperament and Character Inventory – Revised. We found that applied mental stressors were suitable to evoke a shift in dynamic autonomic sympathovagal balance assessed by linear and nonlinear HRV analysis. The nonlinear methods of HRV analysis revealed associations between personality traits and cardiac autonomic regulation in young healthy subjects: significant negative correlation between harm avoidance and parameter 0V% (index of beta-adrenergic cardiac regulation) during mental arithmetic task and significant positive correlation between cooperativeness and resting measures of HRV complexity - normalized index of complexity (NCI) and normalized index of unpredictability (NUPI). This study could help to understand the interaction between personality characteristics, autonomic nervous system, and risk of cardiovascular diseases.

References


EFFECTS OF GUT MICROBIOTA MANIPULATION BY ANTIBIOTICS ON PLASMA AMINO ACID LEVELS IN OBESE HUMANS

Evelien P.J.G. Neis
E-mail: e.neis@maastrichtuniversity.nl

Department of General Surgery, Maastricht University Medical Center* in Maastricht, The Netherlands

Co-authors: D. Reijnders, G. Goossens, E. Blaak, S. Rensen

Tutor: Cornelis H.C. Dejong

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LOSS OF B CELLS AND THEIR PRECURSORS IS THE MOST CONSTANT FEATURE OF GATA-2 DEFICIENCY IN CHILDHOOD MYELODYSPlastic SYNDROME

Michaela Nováková
E-mail: michaela_novakova@lfmotol.cuni.cz

CLIP - Childhood Leukaemia Investigation Prague, Department of Paediatric Haematology and Oncology, Second Faculty of Medicine, Charles University, Prague, Czech Republic


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FAST ULTRA-HIGH RESOLUTION 3D PROTON MR SPECTROSCOPIC IMAGING OF HUMAN BRAIN AT HIGH MAGNETIC FIELDS

Michal Považan
E-mail: michal.povazan@meduniwien.ac.at

High Field MR Center, Department of Biomedical Imaging and Image-guided Therapy, Medical University of Vienna, Austria

Co-authors: G. Hangel, B. Strasser, E. Hečková, S. Gruber, S. Trattnig, W. Bogner
Tutor: Wolfgang Bogner

Introduction
Magnetic resonance imaging (MRI) is one of the most common imaging modalities nowadays. Another application is a so-called magnetic resonance spectroscopy (MRS), where the MR spectrum is acquired from a localized volume of the body to provide additional biochemical information. The application of MRS and spectroscopic imaging (MRSI) as an adjunct to conventional MRI in clinical practice has been hindered by many factors. One of these factors is a low sensitivity, which may be improved by utilizing higher magnetic fields (>3T). However the transition to higher fields introduces many challenges such as B0/B1 inhomogeneities, chemical shift displacement errors (CSDEs), and shortened T2 relaxation times. In addition, an increase in RF power deposition leads to increased demands on hardware as well as software of the MR scanner. Therefore, novel MRSI techniques based on free induction decay (FID) acquisition were introduced for ultra-high field (UHF, ≥7T)\(^1\)\(^2\). Without any pre-localization and without the necessary power that is usually needed to suppress the unwanted signals of lipids, the lipid contamination posed a severe problem. To overcome this, we used high resolutions up to 23 ml and 128×128 matrix sizes in order to achieve a good point spread function and thus prevent the lipid signal from contaminating the brain region. Yet, for such resolutions, typical measurement times range from 30 min up to 2 hours - an unbearable time for any patient measurements. We therefore decreased the acquisition time significantly by acceleration using parallel imaging (PI). Reaching resolutions similar to conventional MRI at a clinically feasible time (~6-17 min) enables the analysis of smaller structures and lesions, which offers a high potential for research as well as for diagnostic applications.

Methods
Volunteers, tumor patients and MS patients underwent a measurement on a Siemens 7 T Magnetom scanner with a 32-channel head coil with different protocols. Written informed consent and IRB approval were obtained. All sequences were measured with an acquisition delay (TE*) of 1.3 ms, an FOV of 220×220 mm\(^2\) and a slice thickness ranging from 6-12 mm. The signals from individual coil channels were combined using an in-house developed method (MUSICAL)\(^3\). To achieve a clinically feasible protocol, acceleration methods based on various undersampling patterns (GRAPPA\(^4\), 2D- and (2+1)D-CAIPIRINHA\(^5\)) were implemented and compared. The data from multiple voxels (often >10 000) per one volunteer were acquired, hence a fully automated processing pipeline with minimal user interaction was implemented. To reduce contaminant lipid signal from skull we utilized a regularized reconstruction according to Bilgic et al.\(^6\) The LCModel quantification software was used for data processing. We adapted the sequence to different applications: 1. A double-inversion recovery sequence to obtain the macromolecular background\(^7\) and to suppress skull lipids\(^8\) for cases where the regularized reconstruction might not be enough. 2. For cases where bigger 3D-coverage was necessary, such
as tumor patients, pulse-cascaded Hadamard encoding together with \((2+1)\)D-CAIPIRINHA were implemented. In this case, each additional Hadamard encoded slice had \(1\) ms increased \(T E^*\). If very small lesions had to be measured, e.g. in MS patients, resolutions up to \(128\times128\) were used (ultra-high resolution, UHR). In this case, acceleration was achieved by reducing the TR from 600 ms to 200 ms by optimizing the water suppression and the read-out module, in addition to 2D-CAIPIRINHA\(^9\).

**Results**

\((2+1)\)D-CAIPIRINHA had smaller root mean square errors in comparison to 2D-GRAPPA (12.1 \%) and 2D-CAIPIRINHA (6.9 \%) with respect to the gold standard without any acceleration. Figure 1 shows metabolic maps obtained from a healthy volunteer. The higher resolution (128x128 matrix) allow to resolve a gray matter and white matter separation and even finer structures such as gyri (Figure 2). An average SNR for UHR was 24\(\pm\)8 over 3 volunteers compared to 24\(\pm\)8 (TR=200ms, 64x64, R=4) and 40\(\pm\)12 (TR=600ms, 64x64, R=4). Reducing the spectral sampling points affected CRLBs and FWHMs more than the smaller voxel volume, with average CRLB[%]/FWHMs[Hz] of 16\(\pm\)7/10\(\pm\)5 for UHR-MRSI. The qualitative assessment of fourteen MS patient data showed a decrease of (25.8\(\pm\)13.4\%) of total signal of N-acetyl aspartate, and (2.3\(\pm\)15.7\%) of total signal of creatine, while inositol increased by (41.8\(\pm\)34.2\%) and total signal of choline by (2.3\(\pm\)16.5\%). In three patients, metabolic changes were visually not or only hardly seen on the 64x64 resolution maps, but were clearly visible on the UHR-MRSI (Figure 3).

**Discussion and conclusion**

We have successfully shown that fast MRSI with higher resolution in the brain at 7 T is feasible within a reasonable time for healthy subjects as well as patients. MRSI acquisition is always a trade-off between sufficient SNR and measurement time. By careful optimization of MRSI parameters, we are able to acquire high-resolution data in less than 20 minutes covering a large area of brain.

**Summary**

Since the existence of magnetic resonance imaging as a clinical imaging modality, the MR spectroscopy protocols were present on the MR scanners offering additional biochemical information from the tissue of interest. However, due to many limitations, MR spectroscopy is considered rather a powerful scientific tool than a robust method used in an everyday clinical practice. Therefore one of the aims of MR spectroscopists is to develop a protocol applicable in the day-to-day patient measurements in any hospital possessing an MR scanner. We tried to optimize the MR spectroscopic imaging sequence for various applications by utilization of available advanced methods of acceleration and data reconstruction. Afterwards, this sequence was tested on healthy volunteers, MS patients and tumor patients to assess the feasibility. We hope that we did another tiny step towards the utilization of MR spectroscopy in clinical routine.

**References**


Figures:

**Figure 1.** $T_1$-weighted image and three metabolic maps acquired from a healthy volunteer
Figure 2. UHR-maps of one volunteer compared to maps with 64×64 matrix size and the same TR of 200 ms and TR of 600 ms. Finer anatomical details, especially the gyri structure can be seen on the UHR-maps.

Figure 3. Comparison of a 64x64 and a 100x100 total N-acetyl aspartate (tNAA) map with a FLAIR image. Two lesions are visible on the 100x100 tNAA map, which are hardly visible on the 64x64 image. This demonstrates that a resolution of 64x64 is sometimes not sufficient to resolve smaller MS lesions.
LEAN BODY MASS ESTIMATION FORM STANDARD DIXON BASED ATTENUATION CORRECTION IN INTEGRATED POSITRON EMISSION TOMOGRAPHY/MAGNET RESONANCE IMAGING

Ivo Rausch
E-mail: ivo.rausch@meduniwien.ac.at

Center for Medical Physics and Biomedical Engineering, Medical University of Vienna, Austria

Co-authors: P. Rust, M. DiFranco, M. Lassen, A. Stadlbauer, M. E. Mayerhoefer, M. Hartenbach, M. Hacker and T. Beyer

Tutor: Thomas Beyer

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GENETICS AND FUNCTIONAL ANALYSIS OF CZECH PATIENTS WITH CONGENITAL HYPERINSULINISM

Klára Roženková
E-mail: klara.rozenkova@fnmotol.cz

Department of Paediatrics, 2nd Faculty of Medicine, Charles University and University Hospital Motol, Prague, Czech Republic

Co-authors: L. Dušátková, P. Dušátková, A. Nessa, J. Maliková, B. Obermannová, J. Kytnarová, Z. Šumník, J. Lebl, K. Hussain, Š. Průhová

Tutor: Štěpánka Průhová

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MITOCHONDRIAL FUNCTIONS IN STEATOTIC RAT LIVER

Ondřej Sobotka
E-mail: sobotkao@lfhk.cuni.cz

Department of Physiology, Faculty of Medicine in Hradec Králové, Charles University, Hradec Králové, Czech Republic

Co-authors: O. Kučera, P. Staňková, R. Endlicher, K. Nozičková, A. Banni, Z. Červinková

Tutor: Zuzana Červinková

Introduction
Non-alcoholic fatty liver disease (NAFLD) is a frequent cause of chronic liver disease in western population. This hepatic disorder is characterized by increased accumulation of lipids in hepatocytes and by potential progression to inflammation called steatohepatitis (Loomba and Sanyal, 2013). This process can advance to the liver cirrhosis an eventual end-stage liver failure with a need of liver transplantation. Cirrhosis can be also complicated by the development of hepatocellular carcinoma. The prevalence of NAFLD is more than one-third of all people in western countries with more than 80% prevalence in obese population. NAFLD is mostly linked to lack of physical activity and unhealthy western type of diet (high cholesterol, high saturated fatty acids content). Liver steatosis is often accompanied by other pathological conditions and disorders such as atherosclerosis, metabolic syndrome and type II diabetes. The pathophysiology of NAFLD is associated with insulin resistance, impaired lipid metabolism and increase in reactive oxygen species (ROS) production (Satapati et al., 2015). However, the inflammation is also associated with lipid accumulation and the question of cause and consequence of NAFLD has not been sufficiently answered. The role of the liver mitochondria in NAFLD development and progression has not been fully elucidated yet and studies which would satisfactorily describe the changes in mitochondrial functions over the NAFLD progression are lacking. The vast majority of studies describe some kind of mitochondrial alterations, but facing the critical differences in study designs (type of animal, type and duration of high fat diet, assessment mitochondrial functions) we have to be careful with mitochondrial data interpretation (Kakimoto and Kowaltowski, 2016).

Aim
The aim of this study was to investigate the liver mitochondrial respiration and oxidative stress during high-fat and high-cholesterol diet for 1 to 6 weeks, which mimics NAFLD in humans.

Methods
Experiments were performed on male Wistar rats fed with a commercially prepared diet (Altromin) with high cholesterol and high fat content (HFD, 70 % of energy from lard enriched by 1.25 % cholesterol) for 1 and 3 weeks. Histological changes of liver tissue were evaluated by Hematoxylin eosin, Masson’s trichrome and Oil red O staining. Liver functions were estimated by measurement of biochemical markers (ALT, AST, ALP, bilirubin, urea) in serum. Triglycerides (TG) and cholesterol were assessed both in serum and in liver homogenates using commercial kits. Mitochondria were isolated from liver by differential centrifugation. Mitochondrial respiration was assessed using OROBOROS Oxygraph 2k with using comparative reference protocols. Tricarboxylic acid cycle, fatty acid oxidation (FAO) and glycerophosphate dehydrogenase were evaluated by titrations of respective substrates and inhibitors.
**Results**
High fat diet did not influence the body weight or body weight gain even after 3 weeks in comparison to low fat diet control. Serum TG, HDL and LDL cholesterol as well as glycaemia were not different between both groups, but we detected progression of liver steatosis already after one week of HFD. Relative liver weight was significantly higher after 3 weeks of HFD feeding. Increased accumulation of lipids and micro vesicular steatosis were present in HFD group after staining by Oil red O and Hematoxylin eosin respectively. We also detected higher content of TG and cholesterol in liver homogenates. Mitochondrial respiration demonstrated significant increase in FAO capacity and relative inhibition of succinate stimulated respiration in HFD group. Maximal mitochondrial respiratory capacity was significantly increased after 3 weeks and mitochondria from HFD group exhibited more efficient oxidative phosphorylation.

**Discussion**
We described the development of early phase of NAFLD in rats fed by HFD. After the first three weeks of experiment we observed no significant biometrical changes even though the energy intake of HFD group was higher by more than 30% of controls. Already after one week of HFD there was a mild grade of fat accumulation in hepatocytes, but we did not observe any signs of impaired lipid metabolism or liver functional damage in serum biochemical markers. Liver mitochondria revealed higher capacity for FAO from the beginning of our study with more pronounced effect after three weeks of HFD. We may thus conclude that mitochondria started an adaptation process to reflect the diet with high fat and high cholesterol content by enhancing their capacity for FAO. To this day we were able to gain the results only from first and third week of the study, but the planned duration is 12 weeks.

**Summary**
We succeeded to induce liver steatosis by HFD in rats already after one week of feeding. We did not detect any changes in body constitutions but we observed higher TG accumulation in hepatocytes and mitochondrial modifications demonstrating increased FAO capacity.

**Acknowledgements**
This work was funded from grants PRVOUK P37/02 and SVV-2016-260287

**References**


CONGENITAL AFIBRINOGENEMIA: NOVEL MUTATION FIBRINOGEN M MARTIN LEADING TO PREMATURE TERMINATION CODON IN FIBRINOGEN B BETA-CHAIN GENE

Tomáš Šimurda
E-mail: tsimurda@orava.sk

National Centre of Hemostasis and Thrombosis, Department of Haematology and Transfusiology, Comenius University in Bratislava, Jessenius Faculty of Medicine in Martin, Slovakia

Co-authors: Z. Snahnicanova, D. Loderer, J. Stasko, Z. Lasabova

Tutor: Peter Kubisz

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MOLECULAR GENETIC ANALYSIS OF ADENOID CYSTIC CARCINOMA OF SALIVARY GLANDS

Petr Šteiner
E-mail: steiner@biop Dicka.cz

Šikl’s Department of Pathology, Charles University, Faculty of Medicine in Pilsen, Czech Republic, and Biop Dická laboratorí s.r.o. Pilsen, Czech Republic

Co-author: L. Hauer

Tutor: Alena Skálová

Introduction
Adenoid cystic carcinoma of the salivary gland (AdCC) is slowly, but steadily growing and recurrent carcinoma which often results in fatal outcome. AdCC is well known for its absence of reliable prognostic markers and successful therapy. Translocation t(6;9)(q22-23;p23-24) resulting in the fusion of MYB and NFIB genes is typical for AdCC. Also, deletion of 1p36 locus can serve as a prognostic marker in various malignancies.

Aim
To identify the frequency of t(6;9)(q22-23;p23-24) and the frequency of 1p36 deletion in a cohort of 27 patients with AdCC and to compare these results with available clinical data.

Methods
Among 1696 salivary gland carcinomas in Registry of Šikl’s Pathology Department in Pilsen, 268 cases of AdCC have been identified. In this study, 27 patients with AdCC, who were treated in Faculty Hospital in Pilsen between years 1986 - 2016, were examined using FISH probes detecting breaks in MYB, NFIB genes, and MYB-NFIB fusion gene, respectively. The presence of fusion transcript MYB-NFIB was detected by RT-PCR. Deletion of locus 1p36 was also examined by FISH. These results were correlated with clinical data.

Results
Break of the MYB gene has been detected in 18/24 (75%) cases, break of the NFIB gene in 20/23 (87%) cases and fusion gene MYB-NFIB has been detected in 19/21 (90%) cases. Fusion transcript of the MYB-NFIB gene has been detected in 5/18 cases. 1p36 locus deletion has been detected in 3/23 (13%) cases. Results of this study are summarized in Table 1.

Discussion
Taken together, the MYB-NFIB fusion has been identified in 19/24 (79%) cases. In two cases, break of NFIB was present in absence of break of the MYB gene. This indicates the presence of other fusion partner than MYB. In one of these two cases, break of MYBL1 gene has been identified. Fusion partner in second case is currently unknown. The presence of MYB-NFIB fusion in concurrent absence of MYB and NFIB breaks indicates complex chromosomal rearrangement. All of three patients with deletion of 1p36 died of disease soon after diagnosis.

Conclusion
Detection of MYB-NFIB fusion can be used as a diagnostic marker of difficult cases of AdCC departing from conventional histomorphology. Considering the fact, that all three patients with 1p36 deletion has died of disease in a short time after diagnosis, the deletion of 1p36 locus...
might be used as an adverse prognostic marker of AdCC. Further research should be accomplished to confirm our findings.

**Summary**

We have confirmed the role of t(6;9)(q22-23;p23-24) as differential diagnostic tool in the diagnosis of AdCC. Deletion of 1p36 was identified as a possible adverse prognostic marker of AdCC. This study was supported by grant SVV 2016 No.260 286.

**Table 1:** Molecular genetic analysis of salivary adenoid cystic carcinomas.

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References


LABEL-FREE QUANTIFICATION OF ENDOGENOUS PEPTIDES RELATED TO INFECTIOUS INFLAMMATION IN PPROM PREGNANCIES

Marie Vajrychová
E-mail: marie.vajrychova@unob.cz

Department of Molecular Pathology and Biology, University of Defense Faculty of Military Health Sciences, Hradec Králové, Czech Republic

Co-authors: V. Tambor, K. Pimková, M. Kacerovský

Tutor: Juraj Lenčo

Introduction

Infectious inflammation caused by a microbial invasion into the amniotic cavity followed by a histological chorioamnionitis (MIAC/HCA) complicates a significant portion of pregnancies with preterm prelabour rupture of the membranes (PPROM). Robust tools for prompt detection of MIAC/HCA are thus required to improve the management of PPROM pregnancies (particularly in low-gestational ages) and to prevent the fetal immune system from developing fetal inflammatory response syndrome (Gotsch et al., 2007). Amniotic fluid is a dynamic milieu closest to fetus, when protein and peptide presence makes it an attractive analytical material for a biomarker discovery using mass spectrometry-based proteomics. Our recent study revealed dysregulation in a large number of amniotic fluid proteins potentially associated with MIAC/HCA in PPROM, including several distinct proteases (Tambor et al., 2012). The up-regulation of proteases suggests that specific endogenous peptides might be generated due to the ongoing pathological process. Hence, the aim of our work was to develop a robust protocol for the enrichment of endogenous peptides from amniotic fluid and apply it for analysis of peptidome changes in PPROM women related to presence of MIAC/HCA.

Methods

The protocol for the endogenous peptides enrichment was based on denaturation of amniotic fluid samples and ultrafiltration. The procedure was exploited to characterize the amniotic fluid peptidome associated with MIAC/HCA in PPROM pregnancies in a cohort of 40 women.

The endogenous peptides were analyzed by a LC-MS/MS using an UltiMate 3000 RSLCnano LC combined with a Q-Exactive Plus mass spectrometer (both from Thermo Scientific). Obtained data were processed using Proteome Discoverer (Thermo Scientific) with integrated algorithm Peakjuggler for label-free quantification. Statistical evaluation was accomplished using program R and Perseus.

Results and Discussion

More than 8000 distinct amniotic fluid endogenous peptides were identified during the LC-MS/MS analyses. The majority of the peptides were generated from collagen chains I and III, implying an ongoing reconstruction of the fetal membranes. A comparative analysis subsequently revealed significant changes in amniotic fluid peptidome due to MIAC/HCA. Endogenous peptides derived from histone H2B protein forms showed the most profound changes. Beyond its role in nucleosome forming, H2B was proved to act as an antimicrobial agent in human placenta (Kim et al., 2002). Similarly, dermcidin peptides dysregulated in the presence of MIAC/HCA were found (Burian and Schittek, 2015). Of note were further peptides resulted from insulin-like growth factor-binding protein that is directly associated with PPROM (Abdelazim, 2014).
Conclusions
More than 8000 endogenous peptides were identified across all amniotic fluid samples, making thus the enrichment protocol viable. Moreover, a portion of endogenous peptides was found to be significantly due to MIAC/HCA, deserving thus more targeted focus.

References
DIFFERENTIAL EXPRESSION OF THE HOMOLOGOUS RECOMBINATION DNA REPAIR GENES IN EARLY AND ADVANCED STAGES OF MYELODYSPLASTIC SYNDROME

Jan Válka
E-mail: jan.valka@uhkt.cz

Institute of Hematology an Blood Transfusion, Praha, Czech Republic

Co-authors: J. Veselá, H. Votavová, A. Jonášová, J. Čermák and M. Beličková

Tutor: Jaroslav Čermák

Background and Aims
The high incidence of mutations in patients with myelodysplastic syndrome (MDS) (1) strongly suggests an implication of defective DNA repair mechanisms (2) in the MDS pathogenesis. Based on the hypothesis that genetic changes are amplified during evolution of MDS-cell clone and disease progression (3); we investigated abnormalities in DNA repair gene expressions and monitored their possible development during MDS progression. Further, we focused on sequencing of selected DNA repair genes and searched for their mutations associated with the disease. (4)

Methods
First, gene expression of 84 DNA repair genes in bone marrow (BM) CD34+ cells of 18 MDS patients was measured by RT² Profiler PCR Arrays (Qiagen). Validation of expression data in selected genes was performed on a cohort of 100 MDS patients. Moreover, paired samples from 15 patients with disease progression were used for monitoring of RAD51 and XRCC2 gene expressions in the course of disease. Mutational analysis was performed on 84 DNA repair genes in 16 patients by targeting next generation sequencing (NGS) (SeqCap EZ System, NimbleGen). Detected mutation was confirmed by Sanger sequencing. Multivariate analysis using a Cox regression model to determine the independent impact of each variable (BM blasts, hemoglobin, neutrophils, platelet count, karyotype, and gene expressions of RAD51 and XRCC2) examined for overall survival (OS) was done.

Results
RAD51 (p<0.0001) and XRCC2 (p<0.0001) genes showed differential expression between low-risk, high-risk patients and control samples (Figure 1A). Patients with high expression level of RAD51 gene had significantly longer OS to those with low level (median: 108.4 vs. 21.9 months; p<0.0001; HR=0.26) (Figure 1B). The expression of RPA3 gene was decreased (FC=-2.65) in all MDS patients (p<0.0001). Down-regulated expression of XRCC2 gene, located on 7q36.1, correlated with monosomy 7 or partial deletion of the long arm of chromosome 7 (7q-) which are associated with a poor prognostic category. Thus, XRCC2 gene may be a candidate leukemogenic gene. Furthermore, expressions of RAD51 and XRCC2 genes were measured in paired samples of patients with disease progression; the expressions were gradually decreased along the stepwise progression (from initial to advanced stages of MDS) (Figure 2). Multivariate analysis identified high expression level of RAD51 gene (HR 0.49; p=0.01) and cytogenetic category (HR 1.68; p=0.002) as significant prognostic factors for OS. Interestingly, overexpression of RAD51 and XRCC2 was observed in patients with advanced disease progression to AML with myelodysplasia with 35-40% of BM blasts. This might indicate the evolution of highly resistant clone with an increased function of DNA repair.
A heterozygous frameshift mutation caused by a deletion of two base pair in codon 263 of \textit{XRCC2} gene (XRCC2: c.789_790delCA) was detected in one patient. CD3+ T-cells were used as control to distinguish germline mutation from somatic one. The result indicated the germline mutation. \textit{XRCC2} expression was significantly down-regulated in this case (FC= -5.04).

**Conclusions and Summary**

Our study demonstrates that an alteration of DNA repair factors, mainly \textit{RAD51} and \textit{XRCC2}, key mediators in the homologous recombination repair of DNA double strand breaks, may contribute to the pathogenesis of MDS. The gene expression of \textit{RAD51} and cytogenetic category were shown to be the strongest independent prognostic factors for OS. The inappropriate function of DNA repair in CD34+ cells seems to be progressive in the course of disease, allowing formation and accumulation of new mutations during MDS clone evolution resulting in development of highly resistant leukemic cells. Notably, we described the new germinal mutation in \textit{XRCC2} repair gene associated with MDS.

Supported by AZV grants (16-33485A and 16-31689A) and the project for conceptual development of research organization (00023736) from the Ministry of Health of the Czech Republic

**Figure 1: \textit{RAD51} gene mRNA expression**

(A) Differential mRNA expression of \textit{RAD51} gene between MDS stages.

(B) Kaplan–Meier curves of overall survival (OS) according to mRNA expression of \textit{RAD51} gene.
Figure 2: *RAD51* mRNA expression in selected MDS patients with the progression.

References


SENECENCE IS MODULATED BY THE UPR RESPONSE IN OVARIAN SURFACE EPITHELIAL CELLS

Kateřina Vašíčková
E-mail: katerina.krat@gmail.com

Department of Histology and Embryology, Faculty of Medicine, Masaryk University in Brno, Czech Republic

Tutor: Petr Vaňhara

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ACUTE LIVER INJURY: ROLE OF EXTRACELLULAR DNA

Lenka Vokálová
E-mail: vokalova@gmail.com

Institute of Physiology, Faculty of Medicine, Comenius University, Bratislava, Slovakia

Co-authors: L. Lauková, J. Čonka, V. Melišková, V. Borbělyová, J. Bábičková, P. Celec

Tutor: Daniela Ostatníková

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EXPLORING THE ROLE OF METALS IN WOUND REPAIR

Holly Wilkinson
E-mail: Holly.wilkinson@hull.ac.uk

School of Life Sciences, The University of Hull, United Kingdom

Co-authors: K. Theis, P. Lythgoe, K. Mace, J. Greenman

Tutor: Matthew Hardman

Introduction

In response to damage our skin invokes a highly dynamic repair programme. Essential for effective repair is activation of the innate immune response, where a diverse range of immune cells orchestrate healing and remove unwanted pathogens. Co-ordinated temporo-spatial regulation of keratinocyte, fibroblast and endothelial cell function leads to wound closure, restoration of dermal tissue and generation of new blood vessels (Broughton et al., 2006). While a plethora of studies have identified genomic, transcriptional and even epigenetic regulators of healing, few studies to date have explored the role of global metal profiles in the events leading to skin repair (Lansdown et al., 2007).

Metal ions and metal complexes are ubiquitous throughout the body, where they control diverse cellular signalling pathways. Indeed, alterations in the global tissue distribution of metals, known as the metallome, results in diabetes, cardiovascular, neurodegenerative and a host of other disorders (Carboni et al., 2015). These chronic degenerative conditions share defective cellular processes also linked to wound pathology, e.g. immune cell dysfunction (Elsholz et al., 2014). Pathological wound healing remains a major area of clinical unmet need. Chronic, non-healing wounds, prevalent in the elderly and diabetic, cause increased patient morbidity and significant financial costs to healthcare providers. Current treatments for chronic wounds are inadequate, often addressing secondary symptoms rather than the primary cause (Percival et al., 2015).

At the tissue level chronic wounds are thought to be the result of defects to one or more cellular aspects of wound repair, however, much remains to be elucidated (Walsh et al., 2016).

Here, we hypothesised that changes in the metallome play an important role in pathological wound healing. Thus, the aims of this project are to profile metals in normal and pathological wound healing, and functionally test the effects of manipulating specific metals during wound repair.

Methods

In vivo wounding. Female pathological healing (20 month old “aged” C57/BL6 and diabetic LEPR-/-) and normal healing (2 month old “young” C57/BL6 and lean LEPR -/+) control mice were anaesthetised and two full thickness 6mm excisional wounds made on the dorsum using sterile trace metal free titanium instruments. Wounds (n=3-5) were collected at 1, 3 and 7 days post-wounding, bisected and processed for subsequent analysis.

Inductively coupled plasma mass spectrometry (ICP-MS). Samples were freeze-dried, digested overnight in trace metal free nitric acid/hydrogen peroxide with a certified reference material (DOLT-5 dogfish liver), and analysed using an Agilent 7500cx.

Histological analysis. Paraffin -embedded samples were sectioned at 5µm. Haematoxylin and eosin (H&E) stained slides were used for wound measurements and Alizarin Red was used to visualise Ca.

qPCR. RNA was extracted (TRIzol® reagent), reverse transcribed and amplified using MESA GREEN. Keratinocyte culture and wounding. HaCaT human keratinocytes were cultured in DMEM with 10% foetal bovine serum (FBS) and 1% penicillin/streptomycin (P/S). Scratch wounds were performed on confluent cells using sterile 1ml pipette tips, stained with crystal violet at 24 and 48 hours post-scratching, and imaged microscopically.

Macrophage culture and activation. Mononuclear phagocyte progenitor cells
were isolated from mouse bone marrow and cultured in DMEM with 10% FBS and 1% P/S. Differentiation was induced using L929 cell conditioned media. Macrophages were M1-polarised by stimulation with LPS and IFN-γ or M2-polarised by stimulation with anti-IFN-γ and IL-4. 

*In vitro metal treatments.* Metals were added directly to cell culture media at final concentrations ranging between 0.01mM and 10mM. *Statistical analysis.* One or Two-Way ANOVA with post-hoc analysis was performed as appropriate.

**Results**

Metallome profiling of normal and pathological repair. Recent methodological advances now permit highly sensitive tissue-specific quantification of metal ions (Dlouhy and Outten, 2013). In this study, ICP-MS profiling revealed that a number of metal species were temporally regulated during normal healing. Next, we profiled the metallome of two different mouse pathological healing models: A) 20 month old “aged” mice with corresponding 2 month old “young” controls and; B) diabetic (LEPR−/−) mice with corresponding lean (LEPR−/+ ) controls. Crucially, profiling metals in skin and wound tissue from these pre-clinical pathological wound models revealed major changes in the local metallome versus controls (Figure 1). Calcium, the most abundant metal in skin, was strongly induced upon acute wounding (P<0.01) yet substantially reduced in both chronic wound models (P<0.05; Figure 1). Profiling expression of calcium-regulated genes confirmed these observations. For example, calmodulin (*Calm1*) was strongly down-regulated in the wounds of aged mice but not young controls. *Functional modulation of metal ions.* *In vitro* studies revealed that exogenous treatment with specific metals directly modulates intrinsic cellular functions required for efficient wound repair. Calcium administration dose-dependently promoted keratinocyte scratch-wound closure (Figure 2). Moreover, calcium treatment effectively dampened pro-inflammatory M1 macrophage polarisation, promoting a tissue resolutory phenotype (Figure 3). Of note, the beneficial effects of calcium on M2 macrophage polarisation were attenuated in cells derived from chronic wound models (Figure 3).

**Discussion**

The majority of research pertaining to metal abundance within the skin is restricted to assessments of bioaccumulation following treatment or occupational exposure (Bianco et al., 2015). Only one prior study has attempted to characterise endogenous metal concentrations in wound tissue. Using flame atomic spectrophotometry on rat wounds, Lansdown et al., (1999) reported changes in zinc, copper, magnesium and calcium throughout normal healing. Here, using the far more sensitive technique of ICP-MS we demonstrate changes in a number of additional metals, and crucially show that metals are altered in murine pathological healing models. In the case of calcium, we note with interest that calcium-regulated genes follow the ICP-MS profile for the metal ion. One such gene, *Calm1*, encodes the cytosolic Ca^{2+}-binding protein calmodulin. Intriguingly, binding of calmodulin to plectin 1a in keratinocytes is important for integrin phosphorylation, thus enabling cell motility (Kostan et al, 2009).

**Conclusions**

These studies demonstrate clear and significant changes in the skin metallome across normal wound repair. Altered levels of specific metals in mouse chronic wounds suggest a direct association with wound pathology, while *in vitro* studies demonstrate direct effects on cellular functions important in wound repair.

**Summary**

Metal ions and metal-containing compounds are widespread in the environment and are essential for life on Earth. For the first time, our results illustrate fascinating changes in the metallome of normal and chronic wounds, and link these to functional changes in skin cells following exogenous metal treatments. These data now provide an unprecedented opportunity to elucidate the crucial roles metals play in the orchestrated wound repair response. Future studies will ascertain
the temporo-spatial distribution of key metal species during acute and pathological repair, and explore the mechanistic links to healing regulation. Ultimately, findings from my PhD should aid the development of novel therapeutics for chronic wounds.

Figure 1. ICP-MS (inductively coupled plasma mass spectrometry) revealing substantial changes in the metal profile of delayed healing wounds. Representative fold change in the global profile of metals between young and aged (Y-axis) and lean and diabetic (Db, X-axis) skin (A) and wounds (B). Illustrative macroscopic wound images are also given (C), where the black, dotted line illustrates overall wound size. Metal sphere size demonstrates the abundance of each metal in a normal-healing young murine mouse model.

Figure 2. Modulation of calcium on human keratinocyte (HaCaT) scratch migration in vitro. Calcium treatment of HaCaTs (A) with representative scratch migration images (4X magnification) at 24 hours (B) and 48 hours (C). Black arrows depict “wound” edges (B and C). Mean +/- SEM, n=4 per group, bar = 500µm (B, C), * = P<0.05, ** = P<0.01 and *** = P<0.001.
Figure 3. Calcium alters macrophage polarisation in normal and pathological healing. Relative expression of macrophage polarisation markers is shown for young (A and B) and aged (C and D) models. Classical activation (M1) is illustrated by pro-inflammatory Nos2 expression (A and C), while alternative activation (M2) is demonstrated by anti-inflammatory Ym1 expression (B and D). “M0”, “M1” and “M2” represent non-polarised, M1-polarised and M2-polarised macrophages, respectively. Macrophages were treated with no calcium (no metal), 0.1mM and 1mM calcium. Calcium treatment significantly dampened pro-inflammatory responses in young macrophages. ND = not done. Mean +/- SEM, n=3 per treatment group, * = P<0.05, ** = P<0.01 and *** = P<0.001.

References


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